PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 26 August 15, 1940 Number 8
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# *RADIOACTIVE CARBON AS AN INDICATOR OF CARBON DIOXIDE REDUCTION. IV. THE SYNTHESIS OF ACETIC ACID FROM CARBON DIOXIDE BY CLOSTRIDIUM ACIDI-URICI*

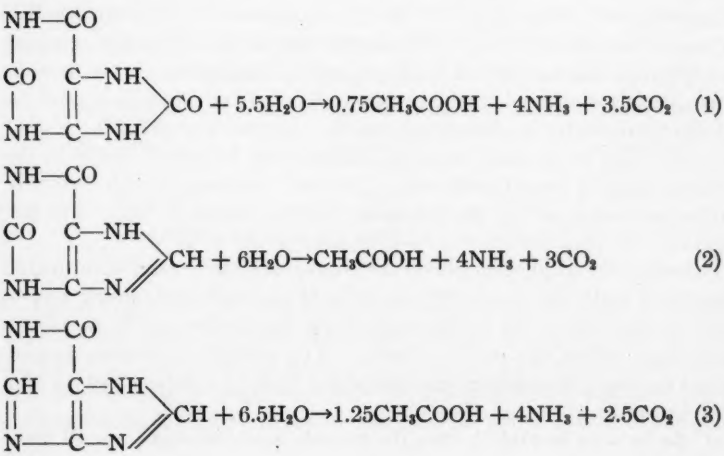
By H. A. Barker, S. Ruben and J. V. Beck

Departments of Chemistry and Plant Nutrition, University of California

Communicated July 11, 1940

*Clostridium acidi-urici* is a little-known organism* capable of rapidly fermenting uric acid, xanthine, hypoxanthine and other purines under strictly anaerobic conditions. Besides cell materials, only three products are formed in considerable quantities in these fermentations, namely, ammonia, carbon dioxide and acetic acid. These substances account for about 98% of the nitrogen and 95% of the carbon of the purines decomposed.

The relative quantities of the fermentation products depend, of course, upon the particular compound used. Uric acid, xanthine and hypoxanthine, for example, are fermented approximately according to the foling equations:



Actually the observed yields of acetic acid are a little lower than indicated by these equations. With uric acid the observed yields in moles of acetic acid per mole of purine fermented have been 0.6–0.7, with xanthine 0.9–1.0 and with hypoxanthine 1.1–1.25.

Now the yields of acetic acid obtained from uric acid and xanthine are in no way remarkable since these quantities might well be derived from the central $C_3$ chain of these purines. With hypoxanthine, however, the quantity of acetic acid is greater than could be obtained directly from this source. To explain the formation of more than one mole of acetic acid per mole of hypoxanthine it must be postulated that either (1) an intra- or intermolecular condensation involving the $C_3$ chains occurs followed by a splitting into the required number of $C_2$ fragments, or (2) part or all of the acetic acid is built up from the carbon dioxide or other $C_1$ fragments of the purine.

Using radioactive carbon ($C^{11}$) as an indicator it has been possible to test the latter alternative and to show that acetic acid is formed by the reduction of carbon dioxide.

For the experiments reported below, suspensions of *Cl. acidi-urici*, strain 9*a*, were used. The organisms were grown in a medium containing 0.3–0.4% uric acid, 0.1% yeast autolyzate, mineral salts and sodium sulfide or thioglycollate as a reducing agent. The reaction was adjusted to pH 7.4. After 18–24 hours' incubation at 35°C. under strictly anaerobic conditions, the cells were centrifuged, washed and resuspended in $M/10$ phosphate buffer pH 7 containing 0.015% $Na_2S.9H_2O$. Cells derived from 250–1000 cc. of medium were used in each experimental vessel. A brief description of the mode of preparation and use of $C^{11}$ (21 minutes half-life) is given elsewhere.[5]

*Experiment 1.*—The object of the first experiment was to see whether radioactive acetic acid is formed during fermentations of uric acid, guanine and hypoxanthine carried out in the presence of radioactive carbon dioxide. Three vessels were used, each containing 5 cc. of cell suspension plus the substances indicated in column 2 of table 1. Glycine was added to vessels 1 and 2 because it has been found to eliminate an induction period in the decomposition of these compounds. The cells were mixed with their substrates just before adding the radioactive carbon dioxide ($C^*O_2$). The suspensions were then shaken in a $N_2$-$C^*O_2$ atmosphere at 37°C.

Following the incubation period the contents of each vessel were treated with 0.5 g. $NaHCO_3$, 0.5 cc. 50% acetic acid and sufficient sulfuric acid to bring the reaction to pH 1; the mixture was then boiled briefly to remove all residual radioactive carbon dioxide. The volatile acids were then removed by steam distillation, neutralized and tested for radioactivity with a Geiger counter.

It can be seen in table 1 that the volatile acids derived from all three

compounds were highly radioactive. The non-volatile fractions were also active. The non-volatile material derived from the hypoxanthine cells contained only about 5% of the total activity; a considerable part of this can be attributed to an incomplete removal of volatile acids. The non-

TABLE 1

DISTRIBUTION OF RADIOACTIVE CARBON IN PRODUCTS OF PURINE FERMENTATION. (ACTIVITIES ARE EXPRESSED IN ARBITRARY UNITS; ALL VALUES ARE CORRECTED FOR DECAY AND ARE DIRECTLY COMPARABLE)

| VESSEL | SUBSTRATE | INCUBATION TIME, MINUTES | PURINE DECOMPOSED MG. | RADIOACTIVITY VOLATILE ACIDS | RADIOACTIVITY NON-VOLATILE FRACTIONS |
|---|---|---|---|---|---|
| 1 | 30 Mg. Na urate 1 Mg. glycine | 15 | 23 | 2.3 | 1.97 |
| 2 | 20 Mg. guanine 1 Mg. glycine | 39 | 9.6 | 1.35 | ... |
| 3 | 10 Mg. hypoxanthine | 79 | ca3 | 7.9 | 0.45 |

volatile activity from the uric acid cells was much larger, being sufficient to account for 46% of the total reduced C*. Not more than about 10% of this 46%—5% of the total—can be attributed to residual volatile acids. It must be concluded that a considerable quantity of some truly non-volatile material has been formed by a reduction of carbon dioxide.

In order to positively identify the volatile acid fraction, it was subjected to Duclaux distillation; the volatile acid from the uric acid culture was used. Five 20-cc. fractions of distillate were collected from a total initial volume of 110 cc. In table 1 the activities of each fraction, expressed in per cent of the activity in 100 cc. of distillate, are compared with similar data obtained by distillation and titration of pure acetic acid solution. The agreement is sufficiently close to leave no doubt that the volatile acid produced from uric acid is very largely if not entirely acetic acid.

TABLE 2

IDENTIFICATION OF VOLATILE ACID FRACTION BY DUCLAUX DISTILLATION

| VOLUME OF DISTILLATE | UNKNOWN % OF ACTIVITY | ACETIC ACID % OF TITRATION |
|---|---|---|
| 20 | 14.3 | 15.7 |
| 40 | 30.9 | 32.7 |
| 60 | 50.8 | 51.3 |
| 80 | 73.8 | 72.7 |
| 100 | 100 | 100 |

*Experiment 2.*—A second experiment was undertaken to find out whether (1) the large quantity of radioactive non-volatile material formed from uric acid is dependent upon the presence of glycine; (2) the non-volatile fraction is soluble or is associated with the insoluble cell materials; (3) the radioactive carbon from carbon dioxide is in the methyl group of the acetic acid.

The procedure was essentially the same as in the previous experiment. Two vessels were used, each containing 5 cc. of cell suspension. In addition, one contained about 30 mg. of sodium urate and 1 mg. glycine; the other vessel contained urate only. Incubation was at 40°C. for 17 minutes and 65 minutes, respectively. In both vessels 22.8 mg. of uric acid were decomposed.

The distribution of radioactive carbon in various fractions of the fermented media is given in table 3. The fractionation of the contents of vessel 1 (containing glycine) was most complete. Immediately after stopping the incubation the cell suspension was centrifuged sharply to separate the cells from the soluble constituents. Volatile acid and non-volatile fractions were then prepared from both cells and supernatant solution. The suspension of vessel 2 (without glycine) was not centrifuged prior to separating the volatile and non-volatile fractions.

TABLE 3

DISTRIBUTION OF RADIOACTIVE CARBON IN PRODUCTS OF URIC ACID FERMENTATION. (ACTIVITIES ARE EXPRESSED IN ARBITRARY UNITS; ALL VALUES ARE CORRECTED FOR DECAY AND ARE DIRECTLY COMPARABLE)

| FRACTION | VOLATILE | NON-VOLATILE | TOTAL | NON-VOLATILE / TOTAL |
|---|---|---|---|---|
| Vessel 1 (glycine) | | | | |
| Supernatant solution | 17.4 | 8.5 | 25.9 | 0.33 |
| Cells | 1.9 | 4.7 | 6.6 | 0.71 |
| Total | 19.3 | 13.2 | 32.5 | 0.41 |
| Vessel 2 (no glycine) | | | | |
| Total | 22.0 | 11.5 | 33.5 | 0.34 |

Table 3 shows that glycine does not markedly affect the total quantities or ratios of volatile and non-volatile activities. Non-volatile activity is present in both the cell fraction and the supernatant liquid. But although the total non-volatile activity is about twice as great in the liquid (9 cc.) as in the cell fraction (1 cc.), the concentration is much higher in the cells. This suggests that at least a considerable part of the radioactive non-volatile material is a cellular constituent. Further experiments would be required to elucidate the nature of this material.

The acetic acid was decarboxylated in order to find out if carbon from carbon dioxide was present in the methyl group. The volatile acid from vessel 1 was neutralized with $Ba(OH)_2$, evaporated to dryness, finely powdered and heated at 450–500°C. for 20 minutes in a stream of oxygen-free nitrogen. The procedure used by Ardagh, *et al.*,[1] was followed. The resulting acetone was trapped in ice-cold 1*N* KOH and converted into iodoform by oxidation with iodine. The equations for the decarboxylation and oxidation reactions are:

$$Ba(CH_3COO)_2 \rightarrow BaCO_3 + CH_3COCH_3 \quad (4)$$

$$CH_3COCH_3 + 3I_2 + 4OH^- \rightarrow CHI_3 + CH_3COO^- + 3I^- + 3H_2O \quad (5)$$

The iodoform, separated by centrifugation and ether extraction, was found to be radioactive, the activity being sufficient to account for approximately 18% of the total activity of the initial acetic acid. Since only one molecule of iodoform is produced from two molecules of acetic acid, the observed activity is sufficient to account for 70% of the theoretical yield on the assumption that the C* is distributed equally between the methyl and carboxyl groups. This calculated yield is a lower limit since the experiment was not carried out in such a way as to give a quantitative recovery of iodoform. Nevertheless the data constitute proof that an appreciable part of the reduced carbon is present in the methyl group.

*Experiment 3.*—This experiment was designed to give further information on the distribution of radioactive carbon between the methyl and carboxyl groups of acetic acid. The procedure was essentially the same as employed in the latter part of experiment 2 except that more care was exercised in the manipulations and, following decarboxylation, the activity of the residual $BaCO_3$ as well as of the $CHI_3$ was measured.

In this experiment the decarboxylation was allowed to proceed at 450–500°C. for 50 minutes. The yield of acetone was determined, following the iodoform reaction, by back titration with thiosulfate. There was obtained 68.5% of the theoretical yield. The iodoform was separated by centrifugation and exhaustive ether extraction, care being taken to have the aqueous phase alkaline to prevent contamination with acetic acid. The carbon dioxide resulting from decarboxylation was obtained by acidifying the residual $BaCO_3$, heating and collecting the evolved gas in alkali. The radioactivity of an aliquot of the alkali solution was determined.

The result of the experiment was the recovery of 23% of the activity of the acetic acid as iodoform derived from the methyl group and 11.3% as carbon dioxide derived from the carboxyl group. When these figures are corrected for the yield of acetone, they become 33.6 and 16.5%, respectively. From equations 4 and 5 it is evident that one of the two acetate radicals in the decomposed barium acetate is regenerated intact so that only 50% of the C* can be recovered in carbon dioxide and iodoform. The observed recovery ($33.6 + 16.5 = 50.1\%$) is in remarkably good agreement with the theoretical maximum.

As for the distribution of C* it appears that considerably more is in the methyl than in the carboxyl group. Since a preferential reduction of carbon dioxide to a methyl group seems unlikely, the observed distribution suggests the existence of reversible exchange reactions between the carboxyl group and carbon dioxide. If such a reaction occurs, the radioactive

carbon in the carboxyl group will be diluted with carbon dioxide produced by the oxidation of uric acid. This point requires further investigation.

*Discussion.*—Although the above experiments show that carbon dioxide is reduced by *Cl. acidi-urici* to acetic acid in fermentations of purines, they do not prove that all the acetic acid is formed in this way. However, it is not improbable that this is the case. If acetic acid is derived entirely from carbon dioxide these "fermentations" would have to be regarded as *complete oxidations of purines with carbon dioxide acting as the ultimate oxidizing agent.* They would then be entirely analogous to the methane "fermentation" with acetic acid replacing methane as the reduction product. The generalized reaction would be

$$4H_2A + 2CO_2 \rightarrow 4A + CH_3COOH + 2H_2O$$

where $H_2A$ represents the reducing agent, A its oxidation product. This interpretation is not without supporting evidence for it has been shown by Wieringa[2] that another Clostridium species is capable of oxidizing molecular hydrogen by means of carbon dioxide, the latter being reduced to acetic acid. In this connection it will be of interest to study the reduction of radioactive carbon dioxide by other acetic acid producing anaerobes.

In conclusion, it may be pointed out that these experiments furnish another example of the already large group of non-photosynthetic, heterotrophic organisms[6] that are able to reduce carbon dioxide. They also illustrate again the use of bacteria for the preparation of organic compounds containing radioactive carbon.[3, 4]

We are indebted to Dr. M. D. Kamen and Professor E. O. Lawrence for the radioactive carbon.

* An extensive study of this organism will be published in the near future.

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# A COMPARISON OF THE METABOLISM OF IODINE AND OF ELEMENT 85 (EKA-IODINE)*

BY JOSEPH G. HAMILTON AND MAYO H. SOLEY

RADIATION LABORATORY AND DIVISIONS OF MEDICINE AND PHARMACOLOGY AND THYROID CLINIC, UNIVERSITY OF CALIFORNIA

Communicated June 17, 1940

One of the procedures employed for the identification of the recently discovered halogen, eka-iodine (element 85), demonstrated that this new element is accumulated in the thyroid gland in a manner similar to iodine.[1, 2, 3] The following report includes a description of the investigation of the comparative metabolism of iodine and element 85 in guinea pigs.

For many years attempts had been made to discover the missing member of the halogen group. All efforts to isolate element 85 were unsuccessful although extremely sensitive tests were available by which quantities of the order of $10^{-10}$ grams could be detected. Perrier and Segre in 1937[4] prepared and identified element 43 by the transmutation of molybdenum with the aid of the 37-inch Berkeley cyclotron. The amounts of element 43 obtained by this method were too minute to permit an investigation of its macroscopic chemical and physical properties. However, this artificially prepared element is radioactive and this characteristic made it possible to observe indirectly its chemical and physical properties and thereby to prove its identity. Pool and Quill[5] in 1938 stated that they had produced element 61 by the transmutation of neodynium in their cyclotron. These discoveries suggested that element 85 might be prepared in a similar manner. However, Oppenheimer[6] pointed out that to do so would require the transmutation of bismuth by alpha-particles (helium ions) possessing an energy in excess of 20,000,000 electron-volts. For this reason any attempts to produce element 85 had to await the availability of more energetic alpha-particles than could be obtained at the time when elements 43 and 61 were discovered. The recent completion of the 60-inch Berkeley cyclotron, which was designed and constructed by Lawrence and his associates, has made it possible to accelerate alpha-particles to energies exceeding 32,000,000 electron-volts. These extremely energetic nuclear particles were first successfully employed by Corson, MacKenzie and Segre[1] in the preparation of element 85. This new element is radioactive and has a half-life of 7.5 hours, but differs from all the other artificially produced radio-elements in that it emits alpha-particles.

*Preparation of the Radio-Halogens.*—Element 85 was produced by the bombardment of a water-cooled metallic bismuth target with 32,000,000 electron-volt alpha-particles. Approximately 200 mgm. of the irradiated bismuth were scraped from the surface of the target and placed in a small

molybdenum boat which was heated to 400 degrees Centigrade in an evacuated bell jar. The volatized element 85 was collected upon a cold piece of glass suspended above the molten bismuth, dissolved in carbon tetrachloride and finally extracted with 10 cc. of 0.016 *N* sodium thiosulphate containing 4 mgm. of sodium carbonate.

Radio-iodine ($I^{121}$) was prepared by the bombardment of metallic tellurium with 16,000,000 electron-volt deuterons. Following the deuteron bombardment in the cyclotron, the tellurium was removed from the target and transferred to a distilling flask equipped with a long delivery tube. Thirty cubic centimeters of 6 *N* nitric acid were then added to the flask and the mixture was heated in order to distill the liberated radio-iodine into a receiver containing 60 cc. of carbon tetrachloride. After the tellurium had been completely dissolved, the carbon tetrachloride was washed twice to remove the nitric acid which had been distilled over with the radio-iodine. The radio-iodine was removed from the carbon tetrachloride by extraction with 10 cc. of 0.016 *N* sodium thiosulphate. These two radio-halogens were separated from the bismuth and tellurium without the use of a carrier. The approximate quantities prepared for each animal were: $5 \times 10^{-12}$ grams of radio-iodine (1 microcurie)** and $10^{-13}$ grams of element 85 (0.2 microcurie).

*Method of Study.*—The animals used in these experiments were guinea pigs of both sexes from 4 to 5 weeks of age whose weights ranged from 170 to 240 grams and which had been raised under the same conditions. In order to facilitate the collection of excreta, the animals were kept in metabolism cages for the duration of the experiments. Two-thirds of the animals received daily injections of thyrotropic hormone for a week before the radio-halogens were administered. The thyrotropic hormone produced symptoms of thyrotoxicosis and marked hyperplasia of the thyroid gland.

The uptake into the thyroid glands and the rates of excretion of radio-iodine and element 85 were observed in groups of thyrotoxic animals and normal controls. Equal and known quantities of radio-iodine and element 85, which had previously been made isotonic by the addition of sodium chloride, were administered together by subcutaneous injection to six thyrotoxic animals and four normal controls. Four hours later three of the thyrotoxic animals and two of the normal controls were sacrificed. Aliquot fractions of the urine and aqueous extracts of the feces collected during the interval together with the thyroids and samples of muscle, blood, liver and lymph nodes, were obtained for measurement of their content of radio-iodine and of element 85. The remaining three thyrotoxic animals and two normal controls were sacrificed 18 hours after injection, and excreta and samples of tissue were obtained as before. The study was repeated without the measurement of urine and feces in nine thyrotoxic animals and six normal controls. These were sacrificed in groups of three thyrotoxic

animals and two normal controls at the end of four, eighteen and sixty-five hours after administration of radio-iodine and element 85. The results of these experiments are shown in tables 1 and 2 and in figure 1.

The tissues and aliquot portions of the excreta were placed in flat ashing crucibles 4 cm. in diameter. One cubic centimeter of 0.1 *N* sodium hydroxide was added to each sample and the tissues were thoroughly macerated. The samples were then placed on a hot plate and evaporated to dryness at a temperature of 100 degrees Centigrade. This procedure made it possible to secure an even distribution of the material inside the crucibles. Aliquot fractions of the administered solutions of the two radio-halogens were measured out into these dishes together with 1 cc. of 0.1 *N* sodium hydroxide, and the mixture was evaporated to dryness. No corrections

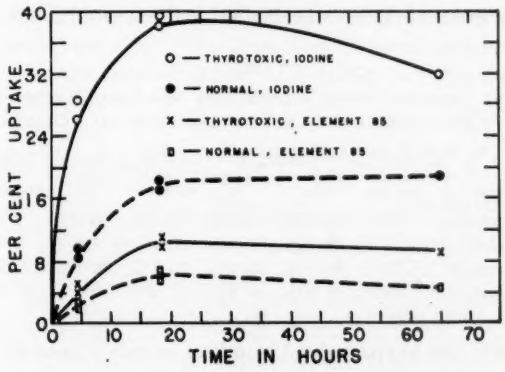


FIGURE 1

The uptake of radio-iodine and element 85 in the thyroid glands of normal and thyrotoxic guinea pigs.

were made for the self-absorption by the tissues and excreta of the radiation emitted by the radio-iodine and element 85 since the dry weight of the samples was less than 2.5 mgm. cm.$^{-2}$. Corrections for the decay of radio-iodine and element 85 in the samples were not made since the standards and samples were measured at the same time.

The content of element 85 in the samples was determined by the use of a shallow ionization chamber which was connected to a linear amplifier and a mechanical recorder. This apparatus made it possible to measure quantitatively the number of alpha-particles emitted by the element 85 in the samples which also contained relatively large quantities of radio-iodine. Since the latter emits beta-particles, its radioactivity did not affect this type of ionization chamber. The radio-iodine in the samples was measured with a DuBridge type of vacuum tube electrometer several

days later in order to allow the element 85 to decay to a negligible quantity. This technique, therefore, made it possible to determine the amount of element 85 and radio-iodine in each sample.

*Results.*—The results recorded in tables 1 and 2 show that element 85 is accumulated in the thyroid gland and excreted in a manner similar to iodine. The curves in figure 1 show that the variations of uptake of radio-iodine and of element 85 by the thyroid glands of the different groups of animals were similar in these experiments although the uptake of the latter was consistently less. The rates of excretion of radio-iodine and element 85 were almost identical and the kidneys apparently acted as the main channel of elimination. The proportion of the two elements excreted in the feces was probably due to contamination from the urine. The contents of the radio-iodine and of element 85 in the other tissues examined was found to be less than 1 per cent of their concentration in the thyroid gland.

TABLE 1

UPTAKE BY THE THYROID GLANDS AND URINARY AND FECAL EXCRETIONS OF RADIO-IODINE AND ELEMENT 85 IN NORMAL AND THYROTOXIC GUINEA PIGS

| ANIMALS | | HOURS AFTER ADMINIS-TRATION | UPTAKE BY THYROID | | URINARY EXCRETION | | FECAL EXCRETION | |
|---|---|---|---|---|---|---|---|---|
| NUMBER | TYPE | | IODINE$^{131}$ | ELEMENT 85 | IODINE$^{131}$ | ELEMENT 85 | IODINE$^{131}$ | ELEMENT 85 |
| 3 | Thyrotoxic | 4 | 26.1% | 4.2% | 13.0% | 16.0% | 1.0% | 0.7% |
| 2 | Normal | 4 | 8.5% | 3.4% | 12.4% | 8.8% | 0.8% | 0.4% |
| 3 | Thyrotoxic | 18 | 38.3% | 10.7% | 25.7% | 34.0% | 5.7% | 8.7% |
| 2 | Normal | 18 | 16.9% | 5.4% | 37.2% | 36.0% | 17.0% | 13.0% |

*Discussion.*—The thyroid gland is unique in that it possesses the ability to accumulate iodine selectively in relatively large quantities. This property is particularly striking because the iodine content of the blood averages less than one part in ten million while the thyroid gland normally contains approximately one part in a thousand. This indicates that the thyroid gland can concentrate the iodine it receives from the blood by a factor of ten thousand. Many organs of the body are capable of storing certain elements, such as phosphorus, magnesium and calcium in the bones, iron in the blood and the liver, and zinc in the pancreas. However, no organ in the body has the power of concentration and storage possessed by the thyroid gland.

A relationship between the periodicity of the chemical and the physical properties of the elements and their physiological action was discovered by James Blake and fully discussed by him in 1848.[7] He studied the effects of nearly all the elements known at that time on the circulation, respiration and the central nervous system of dogs. He was thus able to arrange the elements in groups on the basis of similarity of physiological action. Blake's observations have been confirmed by many investigators both for

the elements available at the time of his experimental studies and for those which have been discovered subsequently.

Since element 85 is a halogen and iodine is its closest homologue, it was felt that further proof of its identity could be secured by a comparison of the biological properties of this new element with iodine. The results of the experiments described in this report show that element 85 shares with iodine the unique property of being selectively accumulated and stored in the thyroid gland. Further proof of the halogen character of this new element is demonstrated by the rapidity of its excretion while all the other heavy elements, such as radium, thorium, lead, bismuth and thallium, tend to be excreted slowly.

The fact that element 85 is retained in the thyroid suggests that it is probably held in firm chemical combination. Many years ago Marine[8] presented clinical data suggesting that once iodine is stored in the thyroid gland its release is very slow. We have recently confirmed his views by directly measuring the rates of uptake and storage of radio-iodine in the

TABLE 2

UPTAKE OF RADIO-IODINE AND ELEMENT 85 BY THE THYROID GLANDS OF NORMAL AND THYROTOXIC GUINEA PIGS

| ANIMALS | | HOURS AFTER | UPTAKE BY THYROID | |
|---|---|---|---|---|
| NUMBER | TYPE | ADMINISTRATION | IODINE$^{131}$ | ELEMENT 85 |
| 3 | Thyrotoxic | 4 | 28.3% | 4.3% |
| 2 | Normal | 4 | 9.3% | 2.9% |
| 3 | Thyrotoxic | 18 | 39.8% | 9.7% |
| 2 | Normal | 18 | 18.5% | 7.1% |
| 3 | Thyrotoxic | 65 | 31.8% | 8.8% |
| 2 | Normal | 65 | 18.8% | 4.5% |

intact thyroid glands of normal human subjects and of patients suffering from various types of thyroid disease.[9, 10, 11] The relatively short half-life of element 85 makes it difficult to follow its metabolism in the thyroid for more than three days. However, the fact that no significant loss from the thyroid glands of either radio-iodine or element 85 was observed during this interval indicates that element 85 is held in thyroid tissue as firmly as iodine.

The increased accumulation of element 85 in the hyperplastic thyroid glands of guinea pigs suggests that this new element may be of potential value in the treatment of human thyroid disorders in which the functional activity of the thyroid gland is abnormally increased. An investigation of the relation between the microscopic anatomy of thyroid tissue and the deposition of radio-iodine has shown that this element is concentrated in the most actively functioning portions of the thyroid glands of goiterous patients.[12] If element 85 is found to behave in a manner similar to radio-iodine in the thyroid of patients with hyperthyroidism, then it should be

superior to radio-iodine as a possible therapeutic agent. This is due to the fact that element 85 emits alpha-particles which have an average energy of 4,000,000 electron-volts, while the beta-rays from the radio-iodine ($I^{131}$) only have an average energy of approximately 200,000 electron-volts. Each alpha-particle gives up its energy in a material such as thyroid tissue within a distance of less than 50 microns, while the beta-rays from radio-iodine lost most of their energy in a distance of 500 microns. The density of ionization produced by these alpha-particles is over two hundred-fold greater than that resulting from the radio-iodine beta-rays. This means that, since the range of the alpha-particles is so short, the thyroid cells which accumulate element 85 in the largest quantities would suffer the brunt of its radiation. The action of radio-iodine in the thyroid gland would be much more diffuse because of the longer range of the beta-rays. Therefore there would be a considerable radiation effect upon the thyroid cells lying at a distance from those areas in which relatively large quantities of radio-iodine have been concentrated.

*Summary.*—A comparison of the metabolism of element 85 and that of iodine has demonstrated that these two halogens are stored in thyroid tissue and are excreted in a similar manner.

*Acknowledgments.*—The authors wish to express their appreciation to Professor Ernest O. Lawrence and his co-workers in the Radiation Laboratory for the preparation of radio-iodine and element 85. The physical measurements were made with the assistance of Drs. Dale R. Corson and Kenneth R. MacKenzie of the Radiation Laboratory. The criticisms and suggestions by Dr. John H. Lawrence of the Division of Medicine, Professor Edwin McMillan and Dr. Emilio Segre of the Radiation Laboratory and Professor Chauncey D. Leake of the Division of Pharmacology are gratefully acknowledged. The experimental animals were supplied by Dr. Herbert M. Evans and his staff of the Institute of Experimental Biology and the thyrotoxic animals were prepared by the administration of a highly purified and potent thyrotropic hormone produced in their laboratory.

* Aided by grants from the John and Mary Markle Foundation and the Christine Breon Fund for Medical Research. Acknowledgment is herewith made for services rendered by the Works Progress Administration, Project No. 10695 and the National Youth Administration.

** 1 microcurie is defined here as the quantity of radioactive material in which $3.7 \times 10^4$ atoms disintegrate per second.

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## *SURFACES OF MINIMAL CAPACITY*

BY G. C. EVANS

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF CALIFORNIA

Communicated June 26, 1940

It is asked whether, given a closed curve $s$ in space, there exists among the surfaces of which $s$ is the complete boundary, one for which the capacity is a minimum. This problem is investigated for the case of a closed curve which is itself of zero capacity and such that there exists a one-one continuous transformation $\xi$ which carries a large sphere, containing $s$ in its interior, into itself in such a way that $s$ is equivalent to a circle $\sigma$, and the points of the surface of the sphere remain invariant. We may regard $\xi$ as extended in the rest of space to be the identical transformation.

If $S$ is a surface bounded by $s$, which except in the neighborhood of $s$ is composed of a finite number of sufficiently smooth pieces, and $V(M)$ is the conductor potential for $S$ it may be shown that a necessary condition for the capacity to be a minimum is the equality

$$\frac{dV(Q)}{dn+} = \frac{dV(Q)}{dn-}, \tag{1}$$

holding for all points $Q$ on the smooth pieces which constitute $S$, $n+$ and $n-$ being oppositely directed normals at the point $Q$. A statement equivalent to (1) is the integral relation

$$\int_S \int \left(\frac{d}{dn_Q}\frac{1}{QP}\right) d\mu(e_P) = 0, \tag{2}$$

where $\mu(e)$ is the distribution of positive mass on $S$ which generates the conductor potential.

The principal theorem is the following:

THEOREM. *There exists a unique surface $S$ bounded by $s$ which satisfies the equation* (1) *at all points of its smooth pieces; and except for nodal lines and points, this surface is analytic.*

In fact, the condition (1) is the condition that $V(M)$ may be extended analytically and as a harmonic function across $S$ in the neighborhood of any smooth piece of it. It may be proved then that $S$ is the whole of the level surface $V = 1$ of this harmonic function.

The proof of the theorem is obtained by setting up this (multiple valued) harmonic function independently. It is necessary to apply the Schwarz alternating process to multiple leaved Riemann manifolds in three dimensions. First we consider the infinite 3-space as bounded internally by a torus about $s$—that is, the image by $\xi$ of a torus about $\sigma$—and introduce the two leaved Riemann manifold into this space in order to obtain a two valued harmonic function which takes on the value 1 on the surface of the torus and with its two branches the values 0 and 2, respectively, at $\infty$.

By letting the torus shrink down to the curve $s$ we obtain a function $v(M)$ with the following properties:

($\alpha$) Either branch of the function is harmonically extensible along any curve in space which does not meet $s$.

($\beta$) The function is bounded and one of its two values tends to 0 at $\infty$, the other to 2.

($\delta$) If the two values $v_1(M)$, $v_2(M)$ of $v$ are distinct at $M$, a closed path which starts from $M$ and loops $s$ once or an odd number of times, returning to $M$, carries $v_1(M)$ into $v_2(M)$, and vice versa, whereas if the closed path does not loop $s$ it carries each value back into itself.

($\lambda$) At every point $M$ not on $s$, $v_1(M) + v_2(M) = 2$.

($\gamma$) Every point of $s$ is a limit point of the level surface $v_1(P) = v_2(P) = 1$.

It may be shown by an extension of Kellogg's fundamental uniqueness theorem[1] that the properties ($\alpha$), ($\beta$), ($\delta$) serve to determine the function $v(M)$ uniquely; of these, ($\alpha$) is required by equation (1), ($\beta$) by the fact that $V(M)$ is the conductor potential and ($\delta$) is an interpretation of the requirement that $S$ be bounded by $s$. Conversely, by these properties, including ($\lambda$) and ($\gamma$), it is seen that the function defined as the minorant of $v_1(M)$, $v_2(M)$ is the conductor potential of $S$ and satisfies (1) at every point. The relation (2) shows that the surface $S$ lies in the convex envelope of the curve $s$.

The problem under discussion is more intimately connected with three dimensions than that of Plateau. The difference in type between the two is in fact made clear by the uniqueness theorem based on ($\alpha$), ($\beta$) and ($\delta$). For instance, if we commence with a plane curve which bounds a figure composed of two circles exterior to each other but connected by a strip, and deform the curve by bending the two circles towards each other, the Plateau figure persists beyond the situation in which a second solution is stable; but in the present problem a nodal point or line must develop, and the figure changes gradually into one in which a surface of the same connectivity as the original one appears as a strip joining the approximately

parallel circumferences of the two circles. Similarly a two-sided figure may be made to change gradually into a one-sided one.

In particular, if $s$ is a plane curve the solution is evidently the portion of the plane enclosed by it. If $s$ lies on a closed convex surface, $S$ is essentially two-sided. The functions $v(M)$, $V(M)$ are in general not necessarily continuous on $s$, for some of the points of $s$ may be irregular boundary points for the conductor potential $V(M)$.

In two dimensions a similar problem has been considered in particular cases as a generalization of a well-known theorem of Koebe on conformal mapping. Pólya and Szegö consider it as a problem in transfinite diameter for a two point boundary in the plane, where the solution is the segment joining them.[2] I am indebted to Professor Szegö for the citations[3] with respect to the problem in the plane. In particular Grötzsch demonstrates by methods of conformal mapping the uniqueness of the solution for an arbitrary finite number of points in the plane.

[1] O. D. Kellogg, "Foundations of Potential Theory," p. 335 (1920).

[2] G. Pólya and G. Szegö, "Transfiniter Durchmesser ebener und räumlicher Punktmengen," *Jour. für die reine und angewandte Mathematik*, **165**, 4–49 (1931).

[3] L. Bieberbach, "Ueber die Koeffizienten derjenigen Potenzreihen, welche eine schlichte Abbildung des Einheitskreises vermitteln," *Akademie der Wissenschaften, Berlin, Zitzungsberichte*, 940–955 (1916); G. Pólya, "Beitrag zur Verallgemeinerung des Verzerrungssatzes auf mehrfach zusammenhängende Gebiete," *Ibid.*, 228–232 and 280–282 (1928–2), 55–62 (1929–2); H. Grötzsch, "Ueber ein Variations problem der konformen Abbildung," *Akademie der Wissenschaften, Leipzig, Berichte*, **82**, 251–263 (1930).

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## *THE POLYNOMIAL OF MITTAG-LEFFLER*

BY H. BATEMAN

NORMAN BRIDGE LABORATORY OF PHYSICS, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated July 13, 1940

1. The polynomial $g_n(z) = 2zF(1-n, 1-z; 2; 2)$ occurs as a coefficient in the expansions

$$(1+t)^z(1-t)^{-z} = 1 + \sum_{n=0}^{\infty} g_n(z)t^n, \quad |t| < 1 \qquad (1)$$

$$2ze^t F(1-z;\ 2;\ -2t) = \sum_{n=1}^{\infty} g_n(z)t^{n-1}/(n-1)! \qquad (2)$$

It was used by Mittag-Leffler[1] in a study of the analytical representation of the integrals and invariants of a linear homogeneous differential equa-

tion in which he made use of a conformal mapping of the $t$-plane on the $w$-plane by means of a relation

$$w = w_0(1 + t)^z(1 - t)^{-z} \tag{3}$$

in which the index $z$ was an imaginary quantity $2b/i\pi$. The first expansion was used later in his researches on the analytical representation of a uniform branch of a monogenic function and was connected with some other expansions. The second expansion is new only in notation being merely a particular case of a well-known expansion in which the coefficients are hypergeometric functions.[2]

Pidduck[3] used an expansion equivalent to (1) in his researches on the propagation of a disturbance in a fluid acted upon by gravity. He gave the recurrence relation

$$g_n(z + 1) - g_{n-1}(z + 1) = g_n(z) + g_{n-1}(z) \tag{4}$$

which is an immediate consequence of the fact that the generating function $G(z, t) = (1 + t)^z(1 - t)^{-z}$ satisfies the functional equation

$$(1 - t)G(z + 1, t) = (1 + t)G(z, t). \tag{5}$$

A second recurrence relation

$$ng_n(z) = (n - 2)g_{n-2}(z) + 2zg_{n-1}(z) \tag{6}$$

has been given by Belorizky;[4] it is a consequence of the fact that

$$(1 - t^2)dG/dt = 2zG. \tag{7}$$

When $|u|$ is sufficiently small the relation

$$\sum_{n=0}^{\infty} u^n(1 + t)^n(1 - t)^{-n} = 1 + 2u\sum_{m=1}^{\infty} t^m(1 + u)^{m-1}(1 - u)^{-m-1} \tag{8}$$

shows that if $n > 0$

$$2u(1 + u)^{n-1}(1 - u)^{-n-1} = \sum_{m=0}^{\infty} u^m g_n(m) \tag{9}$$

and so $2g_m(n + 1) = g_n(m - 1) + 2g_n(m) + g_n(m + 1)$
$= g_{n+1}(m + 1) - g_{n+1}(m - 1).$

Consequently, if $n \geq 1$

$$2g_m(n) = g_n(m + 1) - g_n(m - 1). \tag{10}$$

2. The polynomial $g_n(z)$ may be generalized by writing

$$(1 + t)^{s+r}(1 - t)^{-s} = \sum_{n=0}^{\infty} t^n g_n(z, r). \tag{11}$$

If $(1 + t)^r$ is expanded by the binomial theorem

$$(1 + t)^r = \sum_{n=0}^{\infty} (r/, n)t^n \tag{12}$$

it is found that

$$g_n(z, r) = \sum_{s=0}^{n} (r/, s)g_{n-s}(z), \tag{13}$$

the series terminating earlier when $r$ is a positive integer $< n$.

The expansion (11) is a particular case of the more general expansion of W. Gordon[5] and J. L. Lagrange[6]

$$(1 + t)^{b-c}[1 + (1 - z)t]^{-b} = \sum_{n=0}^{\infty} t^n(-c/, n)F(-n, b; c; z) \qquad |t| < 1, |t - tz| < 1. \tag{14}$$

It follows from this expansion that when $r = 0, 1, \ldots n - 1$

$$g_n(z, r) = (r/, n)F(-n, z; -r; 2). \tag{15}$$

Pidduck considered the case $r = -1$ and so his second coefficient is with a different notation

$$g_n(z + 1, -1) = (-)^n F(-n, z + 1; 1; 2) = F(-n, -z; 1; 2). \tag{16}$$

A second expression for $g_n(z, r)$ in terms of the hypergeometric series is a consequence of Euler's relation

$$F(a, b; c; x) = (1 - x)^{-a} F\left(a, c - b; c; \frac{x}{x - 1}\right) \tag{17}$$

which gives the formula

$$g_n(z, r) = (-)^n(r/, n)F(-n, -z - r; -r; 2). \tag{18}$$

3. The relation

$$F(-n, -z - r; -r; t) = [(z + r/, n)/(r/, n)]\ (-t)^n F(r - n + 1, -n; z + r - n + 1; t^{-1}) \tag{19}$$

indicates also that

$$g_n(z, r) = (z + r/, n)2^n F(r - n + 1, -n; z + r - n + 1; 1/2) \tag{20}$$

and Euler's relation

$$F(a, b; c; x) = (1 - x)^{c-a-b} F(c - a, c - b; c; x) \tag{21}$$

indicates that

$$g_n(z, r) = (z + r/, n)2^{-z} F(z, r + z + 1; r + z - n + 1; 1/2). \tag{22}$$

This formula gives an estimate of $g_n(z, r)$ for large positive values of $n$ when $r + z$ is not one of the numbers $0, 1, 2, \ldots n - 1$ and is also independent of $n$. The hypergeometric series actually gives a convergent expansion in a series of inverse factorials when $g_n(z, r)$ is regarded as a function of $n$. Similarly

$$F(-n, b; c; x) = x^n[(b, n)/(c, n)]\ (-)^n F(1 - n - c, -n; 1 - n - b; z) \tag{23}$$

where $z = 1/x$ and (21) gives for $|x| > 1$

$$F(-n, b; c; x) = x^{b-c}(x-1)^{n+c-b}(b, n)/(c, n)(-)^n F(c - b, 1 - b; 1 - n - b; z). \tag{24}$$

Hence when $|x| > 1$ and $n$ is large

$$F(-n, b; c; x) \sim (-)^n x^{b-c}(x-1)^{n+c-b}(b, n)/(c, n). \tag{25}$$

Negative integral values of $c$ which made $(c, n) = 0$ should be excepted. Also negative integral values of $b - 1$ should be excepted unless $b = c$.

4. Definite integrals for $g_n(z)$ and $g_n(z, r)$ may be derived from the well-known definite integrals for the hypergeometric function. In particular, if $-1 < z < 1$, $n > 0$

$$B(z, 1 - z)g_n(z) = \int_{-1}^{1} t^{n-1}(1 + t)^z(1 - t)^{-z}dt. \tag{26}$$

This result may be written in the alternative form

$$g_n(z) = (1/\pi)\sin(\pi z)\int_{-\infty}^{\infty} e^{uz}(\tanh {}^1/_2u)^n du/sh\ u \tag{27}$$

Differentiating $m$ times with respect to $z$ we find that

$$g_n^{(m)}(z) = \left(\frac{1}{2\pi i}\right)\int_{-\infty}^{\infty}[e^{z(u+i\pi)}(u + i\pi)^m - e^{z(u-i\pi)}(u - i\pi)^m](\tanh {}^1/_2u)^n du/sh\ u \tag{28}$$

Putting $z = 0$ we obtain the formula

$$g_n^{(m)}(0) = \left(\frac{1}{2\pi i}\right)\int_{-\infty}^{\infty}[(u + i\pi)^m - (u - i\pi)^m](\tanh {}^1/_2u)^n\ du/sh\ u \tag{29}$$

for the coefficient of $t^n$ in Mittag-Leffler's expansion

$$[2Q_0(t)]^m = \left[\log\frac{1 + t}{1 - t}\right]^m = \sum_{n=m}^{\infty} t^n g_n^{(m)}(0). \tag{30}$$

When $|R(z)| < 1$ there is a formula

$$g_n(z) = (1/\pi)\int_0^{\pi}(\cot {}^1/_2u)^z \cos({}^1/_2\pi z - nu)du \tag{31}$$

which may be established with the aid of the recurrence relation and may be regarded as holding for negative integral values of $n$ as well as for positive integral values. Since the recurrence relation gives $g_n(z) = 0$ when $n$ is a negative integer we have for all positive integral values of $n$

$$g_n(z) = (2/\pi)\int_0^\pi (\cot {}^1/_2u)^z \cos({}^1/_2\pi z)\cos(nu)du \tag{32}$$

$$g_n(z) = (2/\pi)\int_0^\pi (\cot {}^1/_2u)^s \sin({}^1/_2\pi z)\sin(nu)du \tag{33}$$

If $n > 0$ and $|R(z)| < 1$ the formula

$$g_n(z) = (1/\pi)\int_0^{2\pi} (1 + e^{ia})^z(2 + e^{ia})^{n-1}e^{-ina}da \tag{34}$$

may be derived from the series for $g_n(z)$ in terms of binomial coefficients. A corresponding formula may be obtained for $g_n(z, r)$. Another type of formula for $g_n(z)$ is obtained by starting from the expansion of Liouville[7] and Lerch[8]

$$\exp\left(x\frac{t-1}{t+1}\right) = \sum_{n=0}^{\infty} k_{2n}(x)t^n \tag{35}$$

in which $k_{2n}(x) = e^{-x}(-)^{n-1}(2x)F(1-n;\ 2;\ 2x)$ for $n > 1$
$k_0(x) = e^{-x}$

The formula in question is

$$\Gamma(z)g_n(z) = \int_0^\infty x^{z-1}k_{2n}(x)dx,\ R(z) > -1 \text{ for } n > 0 \tag{36}$$

A corresponding formula for $g_n(z, r)$ is

$$\Gamma(z)g_n(z, -m-1) = (-)^n\int_0^\infty e^{-x}L_n^m(2x)x^{z-1}dx,\ R(z) > 0 \tag{37}$$

where $L_n^m(u)$ is the generalized polynomial of Laguerre. With the notation of Sonine's polynomial

$$g_n(z, -m-1)\Gamma(z) = \Gamma(m+n+1)\int_0^\infty e^{-x}T_m^n(2x)x^{z-1}dx. \tag{38}$$

Another expression for $g_n(z)$ is obtained from the expansion

$$(1-u)^{-2}\exp\left[-x\left(\frac{1+u}{1-u}\right)^2\right] = \sum_{n=0}^{\infty} u^nT_n^*(x)$$

which indicates that

$$\int_0^\infty T_n^*(x)x^{s-1}dx = \frac{\Gamma(z)}{2-4z}(n+1)g_{n+1}(1-2z),\ R(z) > 0 \quad (39)$$

TABLE OF $g_n(m)$

| $n$ | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $m$ | | | | | | | | | | | |
| 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | 1 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 |
| 3 | 1 | 6 | 18 | 38 | 66 | 102 | 146 | 198 | 258 | 326 | 402 |
| 4 | 1 | 8 | 32 | 88 | 192 | 360 | 608 | 952 | 1408 | 1992 | 2720 |
| 5 | 1 | 10 | 50 | 170 | 450 | 1002 | 1970 | 3530 | 5890 | 9290 | 14002 |
| 6 | 1 | 12 | 72 | 292 | 912 | 2364 | 5336 | 10836 | 20256 | 35436 | 58728 |
| 7 | 1 | 14 | 98 | 462 | 1666 | 4942 | 12642 | 28814 | 59906 | 115598 | 209762 |
| 8 | 1 | 16 | 128 | 688 | 2816 | 9424 | 27008 | 68464 | 157184 | 332688 | 658048 |
| 9 | 1 | 18 | 162 | 978 | 4482 | 16722 | 53154 | 148626 | 374274 | 864146 | 1854882 |
| 10 | 1 | 20 | 200 | 1340 | 6800 | 28004 | 97880 | 299660 | 822560 | 2060980 | 4780008 |

$g_0(z) = 1$, $g_1(z) = 2z$, $3g_3(z) = 4z^3 + 2z$, $3g_4(z) = 2z^4 + 4z^2$, $g_2(z) = 2z^2$, $15g_5(z) = 4z^5 + 20z^3 + 6z$, $45g_6(z) = 4z^6 + 40z^4 + 46z^2$.

$$g_n(-z) = (-)^n g_n(z)$$

$$g_{2n}(1/2) = g_{2n+1}(1/2) = \frac{1.3.5\ (2n-1)}{2.4.6.\ 2n} = (n - 1/2, n)$$

[1] Mittag-Leffler, G., *Acta Math.*, **15**, 1–32 (1891); **24**, 183–245 (1901).
[2] Appell, P., *Ann. école norm.*, (2) **9**, 119–144 (1880).
[3] Pidduck, F. B., *Proc. Roy. Soc.* (*London*), **A83**, 347–356 (1910); **86**, 396–405 (1912).
[4] Belorizky, D., *Comptes Rendus*, **195**, 1222–1224 (1932).
[5] Gordon, W., *Ann. der Physik*, (5) **2**, 1031–1056 (1929).
[6] Lagrange, J. L., *Oeuvres*, **2**, 173–234 (1770–1773), see p. 220.
[7] Liouville, J., *J. de Math.*, (2) **2**, 433–440 (1857).
[8] Lerch, M., *J. f. Math.*, **130**, 47–65 (1905).

# BINARY FORMS

BY RUFUS OLDENBURGER

ARMOUR INSTITUTE OF TECHNOLOGY

Communicated June 8, 1940

Elsewhere[1] the author has proved that, for a field $K$ with $(n + 1)$ or more elements, a form $F$ of degree $n$ with a symmetric matrix of coefficients can be written for a finite $\sigma$ as a linear combination of $\sigma$ $n$th powers of linear forms with coefficients in $K$. The existence of such "representations" of $F$ having been established it remains to determine a method of constructing all such representations. In the present note this problem of construction is solved for binary forms by the introduction of certain forms termed "associates." From these results solutions can be obtained for such classical problems as the equivalence and reducibility of forms. The restriction of the treatment to binary forms is not a serious one since most of the theory can be generalized to forms in any number of variables.

1. *Definitions.*—Let $F$ be a binary form of degree $n$ with coefficients in a field $K$ for which the *characteristic* is *greater than* $n$. We can write

$$F = \sum_{i=1}^{\sigma} \lambda_i L_i^n, \tag{1.1}$$

where the $\lambda$'s are elements of $K$, and the $L$'s are linear forms with coefficients in $K$. The sum (1.1) is termed a *$\sigma$-representation* of $F$ with respect to $K$. If $\sigma$ takes on its smallest value in (1.1) for $K$, the integer $\sigma$ is termed the *minimal number* of $F$ for $K$, and the associated *representation* is called *minimal*. The restriction above on the characteristic of $K$ is made throughout this paper so that $F$ will have a unique symmetric matrix of coefficients. Unless the field is specifically mentioned the theorems of this paper are understood to be *valid for fields with this restriction*. We write $F$ as

$$\sum_{1}^{2} a_{ij\ldots k} x_i x_j \ldots x_k,$$

where the indices range over 1, 2, and the matrix $A = (a_{ij\ldots rs\ldots k})$ is completely symmetric. We arrange the elements of $A$ in a 2-way display $\|a_{\alpha\beta}\|$ where $\alpha$ is the set of indices $i, j, \ldots, r$ and $\beta$ the set of indices $s, \ldots, k$ of $A$. In the matrix $\|a_{\alpha\beta}\|$ the indices $i, j, \ldots, r$ vary with the rows of $\|a_{\alpha\beta}\|$ whereas the remaining indices vary with the columns of $\|a_{\alpha\beta}\|$. The rank of $\|a_{\alpha\beta}\|$ is a 2-way rank of $F$ studied elsewhere.[1] We write $F$ in the standard form

$$a_0x^n + na_1x^{n-1}y + \frac{n(n-1)}{2!} a_2x^{n-2}y^2 + \ldots + a_ny^n.$$

We shall make extensive use of the following matrix:

$$A_\sigma = \left\| \begin{matrix} a_0 & a_1 & a_2 & a_3 & \cdots & a_\sigma \\ a_1 & a_2 & a_3 & a_4 & \cdots & a_{\sigma+1} \\ & \cdot & & \cdot & & \cdot \\ a_{n-\sigma} & a_{n-\sigma+1} & a_{n-\sigma+2} & a_{n-\sigma+3} & \cdots & a_n \end{matrix} \right\|.$$

Let $\sigma$ be the number of indices in the set $s, \ldots, k$. The rank of $A_\sigma$ equals the rank of $\|a_{\alpha\beta}\|$. We shall term the rank of $A_\sigma$ the *$\sigma$-rank* of $F$. We shall permit $\sigma$ to take on the value $n$. Let $r$ and $\xi$ denote the vectors

$$\|y^\sigma \quad -y^{\sigma-1}x \quad y^{\sigma-2}x^2 \quad \ldots \quad \pm x^\sigma\|, \qquad \left\| \begin{matrix} \xi_0 \\ \cdot \\ \cdot \\ \cdot \\ \xi_\sigma \end{matrix} \right\|$$

respectively. If the equation

$$\|A_\sigma\|.\xi = 0 \tag{1.2}$$

has a solution for $\xi \neq 0$, the equation defines a form $r\xi$ in $x$ and $y$ up to a constant factor, which form we shall term the *$\sigma$-associate* of $F$. In the case where the 2-rank of a binary cubic is 2, the 2-associate can be taken to be the Hessian of $F$.

*2. Relation between Representations, and the Determination of the Minimal Number.*—By equating coefficients in (1.1) and using various theorems on vectors, we can prove the basic theorem of the present note, which theorem follows.

THEOREM 2.1. *A binary form $F$ of degree $n$ has a representation* (1.1) *with $\sigma \leqq n$ and $s$ of the $L$'s linearly independent in pairs if and only if the $s$-associate of $F$ can be factored into a product of these $L$'s.*

If a $\sigma$-rank of $F$ is $\sigma + 1$ there does not exist a $\sigma$-associate $F_\sigma$ of $F$ in the sense that $F_\sigma \not\equiv 0$. From this and Theorem 2.1 we obtain the following result.

THEOREM 2.2. *The minimal number of a binary form $F$ is the smallest value of $\sigma$ such that the $\sigma$-rank of $F$ is not greater than $\sigma$, and the $\sigma$-associate of $F$ can be factored for some choice of the coefficients of $F_\sigma$ into linear factors, linearly independent in pairs.*

For algebraically closed fields whether or not a binary form has a repeated factor can be recognized by the use of resultants, whence a computation of ranks and resultants yields the minimal number for these fields.

*3. Associates.*—That the associates are intimately related to each other is brought out in the following theorem.

THEOREM 3.1. *If $\rho \geqq \sigma$, and $\sigma$ is such that the $\sigma$-associate $F_\sigma$ of a binary*

*form $F$ exists, the $\rho$-associate $F_\rho$ of $F$ can be factored into $F_\sigma G$ where $G$ is arbitrary.*

The associates of a form $F$ transform covariantly with $F$.

*4. Ranks.*—A study of the matrices of the type $A_\sigma$ of §1 yields the following theorem.

THEOREM 4.1. *For a binary form $F$ of degree $n$ there exists a number $\gamma$ such that the $(n - \sigma)$ and $\sigma$-ranks of $F$ equal $(\sigma + 1)$ for $\sigma = 0, 1, 2, \ldots (\gamma - 1)$ while the remaining 2-way ranks of $F$ equal $\gamma$.*

The smallest value of $\sigma$ for which the $\sigma$-associate of $F$ exists is $\gamma$.

Like the minimal number, the $\sigma$-ranks of $F + \lambda L^n$ differ from the corresponding ranks of $F$ by at most 1. These results have numerous applications to be given elsewhere.

*5. Uniqueness.*—By use of the associates one can show that a $\sigma$-representation of a binary form $F$ is minimal if $\sigma \leqq (n/2) + 1$. This representation is unique in the usual sense if $\sigma \leqq (n + 1)/2$. For the complex field the minimal number of the "general" binary form $F$ of odd degree is $(n + 1)/2$, whence the minimal representation of $F$ is unique. We use "general" here in the sense of Sylvester[2] and Elliott.[3] The present results shed considerable light on the theory of binary forms developed by these men.

If the minimal representation of a form $F$ is unique, the question of its equivalence to another form has a simple answer.

*6. Minimal Number.*—From relations between the minimal number and various factorization properties of $F$ for the complex field it follows that the minimal number of a binary form $F$ attains the value $n$ if and only if $F = L^{n-1}M$ for linear forms $L$ and $M$. That the minimal number does not exceed $n$ was proved elsewhere.[4]

[1] Oldenburger, *Proc. Nat. Acad. Sci.*, **24**, 193–198 (1938).

[2] Sylvester, *Phil. Mag.*, **94**, 391–410 (1851).

[3] Elliott, *Algebra of Quantics*.

[4] Oldenburger and Porges, *Bull. Amer. Math. Soc.*, **5** (1940) (in press).

# SUBGROUPS OF THE GROUPS WHOSE ORDERS ARE BELOW THIRTY

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

Communicated June 19, 1940

A subgroup which is neither the identity nor the entire group $G$ is commonly called a proper subgroup of $G$. In the present article only proper subgroups will be implied by the term subgroup unless the contrary is explicitly stated. The term total number of subgroups will be used whenever the identity and $G$ itself are included among the subgroups of $G$ so that the total number of subgroups of $G$ is always equal to the number of the proper subgroups of $G$ increased by 2 unless $G$ is the identity. There are 88 groups whose orders are below 30 and these furnish many useful illustrations of group properties which can easily be verified on account of the low orders of the groups. They therefore provide a usfeul field for study in view of the fact that the larger groups which are not of prime orders can usually be most conveniently studied by means of their subgroups and have been thus studied since the time of J. L. Lagrange (1736–1813).

Among the general theorems, relating to the number of the subgroups of $G$ the following are especially useful. The cyclic group of order $p^m$,$p$ being any prime number, contains exactly $m - 1$ subgroups and hence there is at least one group which contains an arbitrary given number of subgroups. In the particular cases when this given number is either 5 or 11 it has recently been proved that there is no other group which contains exactly this number of subgroups. The abelian group of order $p^m$ and of the type $m - 1,1$ contains exactly $p + 1$ subgroups of order $p,p^2, \ldots, p^{m-1}$. This is also the case with respect to the non-abelian group which is conformal therewith and exists in all cases when $m > 2$, except when $p = 2$ and $m = 3$. There exists a non-abelian group of order $pq$, $p$ and $q$ being distinct prime numbers and $p > q$, if and only if $p - 1$ is divisible by $q$, and the number of its subgroups is then $p + 1$. When $p - 1$ is not divisible by $q$ the group of order $pq$ contains exactly 2 subgroups and is cyclic.

Suppose that a given group $G$ has exactly $k$ subgroups. It is then possible to construct in the following manner an arbitrary number of groups which separately contain exactly $2k + 2$ subgroups. To do this it is only necessary to find this arbitrary number of prime numbers which are separately prime to the order of $G$ and then to form successively the direct products of $G$ and the separate groups whose orders are equal to these separate prime numbers. For instance, the symmetric group of order 6 contains 4 subgroups and the prime numbers 5, 7, 11, etc., are separately prime to the order of this symmetric group. Hence each of the direct products of

this symmetric group and the group of one of the orders 5, 7, 11, etc., contains exactly 10 subgroups. The resulting groups may clearly be used similarly to construct other infinite systems of groups containing separately exactly $4k + 4 + 2$ subgroups, etc. The entire system thus obtained is composed of abelian or of non-abelian groups as $G$ is either abelian or non-abelian.

The total number of the subgroups of an abelian group whose order is not a power of a prime number is the product of the total numbers of the subgroups in its Sylow subgroups. In particular, the total number of such subgroups cannot be a prime number, and the determination of the total number of the subgroups of any abelian group is reduced to the determination of the subgroups of prime power abelian groups. In the special case when the abelian group is of order $p^m$, and of type $1^m$, $p$ being any prime number, there is a well-known formula which gives the total number of the subgroups directly. In particular, it can be seen from the following lists that among the groups whose orders are below 30 the abelian group of order 16 and of type $1^4$ contains 65 subgroups, which is the largest number of subgroups contained in one of these groups. The generalized dihedral group of order 24 contains 52 subgroups, which is next to the largest number.

*List of the Groups and of the Numbers of Their Subgroups.*—Since a group of prime order contains no proper subgroup these orders are not included in the following list. The only other order below 30 for which there is no non-cyclic group is 15. It is known that a necessary and sufficient condition that there is only one group of a given composite order is that this order is the product of distinct prime numbers such that none of them diminished by unity is divisible by another. The number of the subgroups of such a group is equal to the number of these distinct prime numbers. In the following enumeration of groups only distinct abstract groups are considered while in the enumeration of the subgroups two subgroups are regarded as distinct unless each of them contains exactly the same operators as the other. For instance, the given group which contains 65 subgroups contains only three distinct abstract subgroups; viz., one of each of the orders 2, 4, 8. A similar enumeration with respect to abstract groups would not, in general, be possible, but it has frequently been employed in regard to the permutation groups of a given degree.

Exactly half of the 14 groups of order 16 contain more subgroups than operators. These are the dihedral and the hamiltonian groups of this order, each of which contains exactly 17 subgroups; the abelian groups of types 2, $1^2$ and $1^4$, respectively, which contain 25 and 65 subgroups, respectively; the generalized dihedral group which contains the abelian group of type 2,1 and involves exactly 33 subgroups; the group whose commutator subgroup of order 2 is not found in one of its cyclic subgroups of order 4, and the group whose central is a cyclic subgroup of order 4 but which in-

volves no operator of a larger order. Each of these two groups contains exactly 21 subgroups. The study of the various types of subgroups, such as invariant subgroups and commutator subgroups, has thus far received much more attention than the enumeration of the subgroups, but this enumeration clearly also throws light on the properties of the groups concerned and hence it may be permanently useful just as the enumeration of the possible groups of low orders has been. Important early contributions to the latter enumeration were made by A. L. Cauchy (1789–1857), J. A. Serret (1819–1885), A. Cayley (1821–1895) and others.

LIST OF THE GROUPS AND OF THE NUMBERS OF THEIR SUBGROUPS

| ORDERS | DESCRIPTION OF THE GROUPS OF THE SAME ORDERS | NUMBERS |
|---|---|---|
| 4 | Cyclic, non-cyclic | 1, 3 |
| 6 | Cyclic, non-cyclic | 2, 4 |
| 8 | Cyclic, octic, quaternion | 2, 8, 4 |
| | Type 2, 1; type $1^3$ | 6, 14 |
| 9 | Cyclic, non-cyclic | 1, 4 |
| 10 | Cyclic, non-cyclic | 2, 6 |
| 12 | Cyclic, tetrahedral, dihedral | 4, 8, 14 |
| | Dicyclic, non-cyclic abelian | 6, 8 |
| 14 | Cyclic, dihedral | 2, 8 |
| 15 | Cyclic | 2 |
| 16 | Cyclic, dicyclic, dihedral, hamiltonian | 3, 9, 17, 17 |
| | Type 3, 1; type $2^2$; type 2, $1^2$; type $1^4$ | 9, 13, 25, 65 |
| | Non-abelian conformal with type 3, 1 | 9 |
| | Operator of order 8 and its third powers | 13 |
| | Generalized dihedral involving type 2, 1 | 33 |
| | The commutator of order 2 is not a square | 21 |
| | Twelve operators of order 4, different squares | 13 |
| | Invariant maximal operator of order 4 | 21 |
| 18 | Cyclic, non-cyclic abelian, dihedral | 4, 10, 14 |
| | Generalized dihedral, central order 3 | 26, 12 |
| 20 | Cyclic, non-cyclic abelian, dihedral | 4, 8, 20 |
| | Dicyclic, metacyclic | 8, 12 |
| 21 | Cyclic, non-cyclic | 2, 8 |
| 22 | Cyclic, dihedral | 2, 12 |
| 24 | Direct products abelian Sylow subgroups | 6, 14, 30 |
| | Direct products, non-abelian orders 8 | 18, 10 |
| | Non-twelve, direct product tetrahedral and 2 | 13, 24 |
| | Symmetric, dihedral, dicyclic | 28, 32, 16 |
| | Generalized dihedral, generalized dicyclic | 52, 24 |
| | Operator order 12 and its fifth powers | 24, 8 |
| | Non-cyclic abelian 12, octic and symmetric 6 | 28 |
| 25 | Cyclic, non-cyclic | 1, 6 |
| 26 | Cyclic, dihedral | 2; 14 |
| 27 | Type 3; type 2, 1; type $1^3$ | 2, 8, 26 |
| | Non-abelian conformal with $1^3$, with 2, 1 | 17, 8 |
| 28 | Cyclic, non-cyclic abelian, dihedral, dicyclic | 4, 8, 26, 10 |

# A GENERAL THEOREM ON CONFORMAL MAPPING OF MULTIPLY CONNECTED DOMAINS

BY R. COURANT, B. MANEL AND M. SHIFFMAN

DEPARTMENT OF MATHEMATICS, NEW YORK UNIVERSITY AND COLLEGE OF THE CITY OF NEW YORK

Communicated June 17, 1940

*1. Introduction.*—In the theory of conformal mapping of $k$-fold connected domains the basic problem is to find types of domains $B$ which depend on a finite number of parameters (essentially equal to the number of moduli) such that any $k$-fold connected domain is conformally equivalent to one of them. Such a class of domains $B$ will be called a *class of normal domains*. Methods developed for the solution of Plateau's and Douglas' problem have opened a new approach to this mapping problem. The present note, which depends on previous publications,[1] shows on this basis that a very wide variety in the choice of normal domains is possible.

THEOREM: *The domains consisting of the exterior of $k$ non-intersecting curves which are respectively similar under expansions and translations*[2] *to any $k$ given convex analytic curves form a class of normal domains.*

*Proof.*—Let $G$ be an arbitrary domain in the $(x, y)$-plane bounded by $k$ non-intersecting Jordan curves $\Gamma_j$, $j = 1, 2, \ldots, k$. Without restricting the generality we may assume that $G$ is bounded. It is required to map $G$ conformally on a domain $B$ of the $(u, v)$-plane with boundaries $C_j$, $j = 1, 2, \ldots, k$, of the type stated in the theorem. The mapping under consideration will be given by the functions $x(u, v)$, $y(u, v)$, the components of a vector function $\mathfrak{x}(u, v)$. The function $\mathfrak{x}(u, v)$ and the domain $B$ are characterized as the solution of the following minimum problem: In a domain $B$ of our class, consider vectors $\mathfrak{x}(u, v)$ which are continuous in $B + C$ with piecewise continuous derivatives in $B$, map the curves $C_j$ continuously and monotonically on $\Gamma_j$ and have a finite Dirichlet integral,

$$D[\mathfrak{x}] = {}^1/_2 \int_B \int (\mathfrak{x}_u^2 + \mathfrak{x}_v^2) du dv;$$

we seek the absolute minimum of the Dirichlet integral with respect to the vectors $\mathfrak{x}$ and the domains $B$.

That this variational problem has a solution $\mathfrak{x}$, $B$, and that $\mathfrak{x}$ is harmonic in $B$, has been proved in the papers [1], [2]. The only remaining point is to show that the variational conditions imply a conformal mapping of $B$ on $G$; this is equivalent to showing that the analytic function

$$\Phi(w) = (\mathfrak{x}_u - i\mathfrak{x}_v)^2 = \mathfrak{x}_u^2 - \mathfrak{x}_v^2 - 2i\mathfrak{x}_u\mathfrak{x}_v = \left(\frac{d\mathfrak{x}}{dw}\right)^2$$

vanishes identically, where $\mathfrak{k}$ is the analytic vector function with $\mathfrak{X}$ as its real part.

*2. Variational Conditions.*—It will be useful to consider variational conditions not only in the plane of the variable $w = u + iv$ but also in an auxiliary plane of the complex variable $\zeta = re^{i\theta}$.[3] Let $w = w(\zeta)$ be the analytic function that maps the exterior of the unit circle $K$ of the $\zeta$-plane conformally on the exterior of the curve $C_j$ of the $w$-plane so that the point at $\infty$ goes into the point at $\infty$. We have

$$\Phi(w) = \frac{(d\mathfrak{k}/d\zeta)^2}{(dw/d\zeta)^2} = \frac{\Psi(\zeta)}{(dw/d\zeta)^2}. \tag{1}$$

The variation of the boundary representation of $\mathfrak{X}$ in the $\zeta$-plane yields[4]

$$\Im[\zeta^2\Psi(\zeta)] = 0 \text{ on } K \tag{2}$$

where $\Im$ denotes "imaginary part." The variation of the position of $C_j$ by parallel translations in the $w$-plane yields a condition for $\Phi(w)$ which, when transformed into the $\zeta$-plane, becomes

$$\int_K \frac{\Psi(\zeta)}{dw/d\zeta}\, d\zeta = 0. \tag{3}$$

Finally, varying by an expansion in the $w$-plane, we get

$$\Im \int_K \frac{\Psi(\zeta)w(\zeta)}{dw/d\zeta}\, d\zeta = 0. \tag{4}$$

We will express the conditions (2), (3), (4) in terms of the parameter $\theta$ of the unit circle $K$. Setting $\zeta^2\Psi(\zeta) = f(\theta)$ on $K$ and noting that there $\frac{dw}{d\zeta} = \frac{du + idv}{i\zeta d\theta}$ where $du$, $dv$ are differentials on $C_j$, we obtain

$$f(\theta) \text{ is real,} \tag{5}$$

$$\int_K g(\theta)\frac{du}{d\theta}\, d\theta = \int_K g(\theta)\frac{dv}{d\theta}\, d\theta = 0, \tag{6}$$

$$\int_K g(\theta)\left[u\frac{dv}{d\theta} - v\frac{du}{d\theta}\right] d\theta = 0, \tag{7}$$

where $g(\theta) = \frac{f(\theta)}{|dw/d\zeta|^2}$.[5]

By virtue of (6), condition (7) can be extended to

$$\int_K g(\theta)\left[u'\frac{dv'}{d\theta} - v'\frac{du'}{d\theta}\right]d\theta = 0 \qquad (7')$$

where $u' = u - u_0$, $v' = v - v_0$ and $u_0$, $v_0$ are any constants.

*3.* $\Phi(w) \equiv 0$.—As in [1], the proof that $\Phi(w) \equiv 0$ follows by counting its zeros in $B$. We first show that $f(\theta)$ has at least four zeros on $K$. Let $(u_0, v_0)$ be a point interior to $C_j$; then $u'\frac{dv'}{d\theta} - v'\frac{du'}{d\theta} = u'^2\frac{d}{d\theta}\left(\frac{v'}{u'}\right) \geq 0$ since $C_j$ is convex. The condition (7′) shows that $g(\theta)$, and therefore $f(\theta)$, must change sign. Thus, $g(\theta)$ has at least two zeros $\theta = \alpha$, $\theta = \beta$ where $g(\theta)$ changes sign.

We can determine three constants $a, b, c$, not all zero, so that the function

$$h(\theta) = -b\frac{du}{d\theta} + a\frac{dv}{d\theta} - c\left(u\frac{dv}{d\theta} - v\frac{du}{d\theta}\right) = \frac{du}{d\theta}(cv - b) - \frac{dv}{d\theta}(cu - a)$$

vanishes at $\theta = \alpha$ and at $\theta = \beta$. This function $h(\theta)$ is the outer product of the vectors $\left[c\frac{du}{d\theta}, c\frac{dv}{d\theta}\right]$ and $\left[u - \frac{a}{c}, v - \frac{b}{c}\right]$, the former tangent to $C_j$ at the point $(u, v)$, the latter on the line through $(u, v)$ and $\left(\frac{a}{c}, \frac{b}{c}\right)$.[6] Since $C_j$ is convex, there are only two points $(u, v)$ where this outer product vanishes; moreover, $h(\theta)$ changes sign there since the sine of the angle between the two vectors mentioned above changes sign. Now the conditions (6), (7) yield $\int_K g(\theta)\cdot h(\theta)d\theta = 0$; this shows that $g(\theta)$ must change sign at other places besides $\theta = \alpha$ and $\theta = \beta$, for otherwise $g(\theta)\cdot h(\theta)$ would have constant sign. It follows that $g(\theta)$, and therefore $f(\theta)$, has at least four zeros on $K$.

The analytic function $\Phi(w)$ has at least four zeros on $C_j$ by virtue of (1) and the fact that $\frac{dw}{d\zeta}$ is regular and different from zero on $K$. Furthermore, $\Phi(w)$ is regular in $B$ and has a zero of order at least four at $\infty$. For, in the neighborhood of $\infty$,

$$\mathfrak{t} = \mathfrak{A} + \frac{\mathfrak{B}}{w} + \frac{\mathfrak{C}}{w^2} + \cdots\cdots\cdots,$$

$$\frac{d\mathfrak{t}}{dw} = -\frac{\mathfrak{B}}{w^2} + \cdots\cdots\cdots,$$

and

$$\Phi(w) = \left(\frac{d\mathfrak{t}}{dw}\right)^2 = \frac{\mathfrak{B}^2}{w^4} + \cdots\cdots\cdots$$

If $\Phi(w)$ were not identically zero, the number $N$ of its zeros would be given by $N = \sum_{j=1}^{k} N_j$ where the individual terms are

$$N_j = \frac{1}{2\pi i} \oint_{C_{j_\epsilon}} d \log \Phi(w) = \frac{1}{2\pi i} \oint_{K_\epsilon} \left[ d \log f(\theta) - d \log \zeta^2 \left( \frac{dw}{d\zeta} \right)^2 \right]$$

and $K_\epsilon$ is the unit circle modified by arcs circumventing the zeros of $f(\theta)$ on the boundary and protruding outside the unit circle. The sense of integration is such that the exterior of the unit circle remains on the left. Because $f(\theta)$ has at least four zeros on the boundary, and $\zeta^2 \left( \frac{dw}{d\zeta} \right)^2$ has a pole of the second order at $\infty$, we have $N_j \leq -4(1/2) + 2 = 0$. Thus $N \leq 0$ contradicting $N \geq 4$, so that $\Phi(w)$ must be identically zero. q. e. d.

*4. Concluding Remarks.*—It would have been sufficient to show that $N \leq 3$ to arrive at a contradiction. This allows us to normalize the domain $B$. One such normalization is to keep $C_1$ fixed in size and position and allow $C_2$ to vary only by expanding with respect to an interior point. This would give four zeros on $C_j, j \neq 1, 2$, two zeros on $C_2$ and no information on $C_1$. Thus, $N_1 \leq 2$, $N_2 \leq 1$, $N_j \leq 0$ for $j \neq 1, 2$, and finally $N \leq 3$. In this case, $C_1$ need not be convex and $C_2$ need only be star shaped.[7] Another type of normalization is to allow $C_1$, $C_2$, $C_3$ to vary only by expanding with respect to an interior point of each.

Finally, it should be stated that the property of convexity was used in an essential way in the proof although no such restriction is needed in the existence proof for the minimum problem.[8] However, mapping theorems where certain non-convex curves appear have been established on the basis of Plateau's problem in the thesis of B. Manel, [4]. Also included in this thesis are cases where singularities appear on the boundaries of the normal domain.

[1] See the following papers:

[1] R. Courant, "Plateau's Problem and Dirichlet's Principle," *Ann. Math.*, **38**, 679–724 (1937).

[2] R. Courant, "Conformal Mapping of Multiply Connected Domains," *Duke Math. Jour.*, Oct., 814–823 (1939).

[3] R. Courant, "The Existence of Minimal Surfaces of Given Topological Structure," *Acta Math.*, **72**, 51–98 (1940).

See also,

[4] B. Manel, "Conformal Mapping of Multiply Connected Domains on the Basis of Plateau's Problem," doctoral thesis at New York University, June 1939, to be published soon.

Complete references to further literature, in particular the works of Douglas, are found in [3].

[2] I.e., under transformations of the form $x' = ax + b$, $y' = ay + c$.

[3] This procedure is used for elliptical domains in [4].

[4] Variational conditions have been derived in [3].

[5] $\frac{dw}{d\zeta}$ is regular and different from zero on $K$.

[6] If $c = 0$, $h(\theta)$ represents the outer product of the vector $\left[\frac{du}{d\theta}, \frac{dv}{d\theta}\right]$ and the fixed vector $[-a, -b]$; the reasoning still applies.

[7] A curve $C$ is star-shaped if there is a point such that any straight ray issuing from this point intersects $C$ exactly once.

[8] It seems plausible that the question of uniqueness, which is not discussed in this paper, is connected with the condition of convexity.

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## *RIBONUCLEIC ACIDS IN BOTH NUCLEUS AND CYTOPLASM, AND THE FUNCTION OF THE NUCLEOLUS*

BY T. CASPERSSON AND JACK SCHULTZ*

KEMISKA INSTITUTIONEN, KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN, AND WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated July 13, 1940

The material exchanges between nucleus and cytoplasm were studied actively in the early days of cytology, a study which reached its climax in the theory of "trophochromatin."[1] The methods used at that time were the biological stains which were then considered specific, but more detailed work showed that identity of staining reaction by no means implied identical substances. Particularly with the use of the Feulgen reaction for thymonucleic acid, the evidence for a direct transfer of chromosomal material to the cytoplasm broke down.[2] At the center of the discussion was the nucleolus, which in its staining properties resembled some of the cytoplasmic components, and in some cases appeared indeed to be extruded into the cytoplasm. With the recent advances in cytochemical technique, these problems can be studied more critically. The methods of ultra-violet spectrophotometry applied to the cytological material have been particularly useful in the analysis of the occurrence and distribution of the nucleic acids in the cell. As has been pointed out in detail elsewhere,[3] the absorption spectra of the nucleic acids are sufficiently striking—the maximum lying at 2600 Å in the middle ultra-violet, due to the conjugated double bonds of the constituent pyrimidine rings—so that the course of the absorption curves of biological structures can give evidence of the presence in them of these substances.

As is well known the nucleic acids are differentiated into two groups according to the type of carbohydrate:[4] the desoxy ribose, of which the thymonucleic acid characterizing the chromosomes is the type; and the

ribose nucleic acids, to which the nucleic acid of yeast belongs. The absorption spectra, being determined by the nitrogenous constituents, do not differentiate between these two groups; but in conjunction with other tests the location of any of these substances in the cell may be determined by their use. In this way it was shown that the ribose nucleic acids are found in the cytoplasm of rapidly growing tissues in characteristically high amounts.[5] The data to be presented show that these substances are also present in the nucleolus and in high concentration around the nuclear membrane. Thus one of the rôles of the nucleus in cellular syntheses concerns the cytoplasmic nucleic acids, a discussion of which is of obvious relevance to the mode of action of genes.

Our measurements were made upon the egg of the sea urchin, *Psammechinus miliaris*, the spinach root tip periblem cell and the cells of the *Drosophila melanogaster* salivary gland. In the sea urchin we have an egg in which there are no elaborate systems of nutrition but the brunt of the syntheses is born by the egg itself, without any appreciable evidence of endomitosis in the nucleus. The root tip of *Spinacia*, on the other hand, shows clearly the occurrence of endomitosis, Gentcheff and Gustafsson[6] having recently worked over the double reproduction of the chromosomes in the periblem cells of this form. Finally the salivary gland cell allows the study of composition of the nucleolus in relation to the giant chromosomes and, more especially in *Drosophila*, different genetic types may be compared.

For these measurements preparations were made of cells fixed in a lanthanum acetate-acetic acid mixture, pressed between quartz cover slip and slide, with glycerine then allowed to diffuse under the cover slip. In order to obtain such isolated cells from the spinach root tip it was necessary to macerate the root in 45% acetic acid for several days, after which time the individual cells were teased apart, and the precipitation completed in the lanthanum acetate-acetic acid mixture.

The technique of the absorption measurements has been described in detail elsewhere.[7] The suitable places chosen in the relatively homogeneous nucleoli are centered in the ultra-violet microscope illuminated with monochromatic light dispersed by a mirror monochromator from a high-pressure, water-cooled mercury lamp. The light is directed onto the opening of a diaphragm in front of a photoelectric cell by means of an adjustable quartz prism and the current measured by a string electrometer of the Lutz-Edelman type. The object is then moved by means of a precision mechanical stage, until a neighboring free space is at the center of the field. Now by means of a variable rotating sector the light is cut down until the electrometer reading reaches the same value as the reading when the object was in the field. The percentage of light removed by the sector is then equal to the absorption by the object. The movement of the mechanical stage is sufficiently accurate in the case of relatively large objects like the nucleoli, so that the absorption measurements made at the different wave-lengths are all made at the same place in the object.

The absorption spectra of different regions within the nucleus and cytoplasm of the sea urchin egg are given in figure 1. The course of two of the four curves is evidently determined by the contained nucleic acids. The absorption spectrum of the nucleolus shows a typical absorption maximum at 2600 Å, with a subsidiary hump around 2800 Å, indicating the probable presence of proteins containing the aromatic amino acids such as tyrosine

and tryptophane. A similar absorption curve is given by the cytoplasm around the nuclear membrane, with the difference that there is considerably more absorption due to the protein. The curve for the more peripheral cytoplasm is quite different; there is a high absorption in the short wave-lengths which sinks to a hump around 2600 Å beyond which there is a second lower plateau around 2800 Å. Finally to complete the picture, the

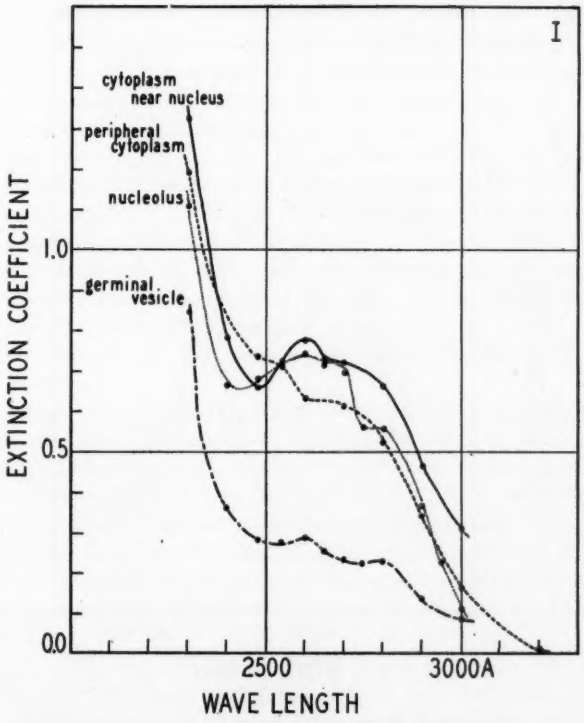


FIGURE 1

Ultra-violet absorption spectra of different parts of an ovarian egg of the sea urchin, *Psammechinus miliaris*. Measurements taken photoelectrically; optical conditions: objective 2.5, ocular 5 ×.

absorption spectrum of the nuclear sap and extended chromosomes of the germinal vesicle shows a slight rise in the nucleic acid region and a plateau in the region of the "protein" band. Towards the shorter wave-lengths the rise is more rapid than that exhibited by the other curves, the ratio of the absorption at 2300 to the absorption at 2800 Å being 3.7 for the germinal vesicle and only 2.3 for the peripheral cytoplasm. The absorption

curves for the various proteins are largely determined by the constituent amino acids;[3,8] and the protamines first isolated from fish sperm by Miescher contain only amino acids which give a high non-specific absorption at the short wave-lengths.[3] These differences observed in the absorption curves may perhaps be attributed to the presence in the germinal vesicle of the sea urchin of protamine like proteins.

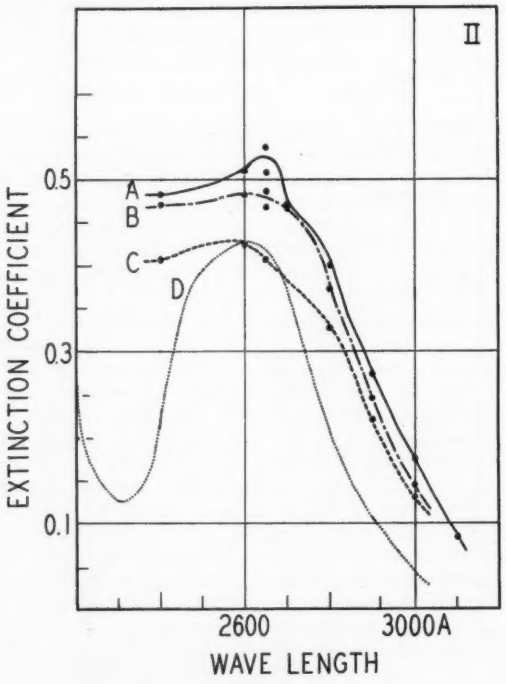


FIGURE 2

Ultra-violet absorption spectra of cytoplasm and nucleolus of *Spinacia oleracea*, var. *Hertha*. Curves *A* and *B*, nucleoli; *C*, cytoplasm of the same cell as *B*; *D*, yeast nucleic acid in concentration corresponding to absorption of *C* at 2600. Optical conditions, objective 2.5, ocular 10 ×, condenser 1.25.

According to Brachet[9] the only Feulgen-positive structures in the sea urchin egg are the chromosome threads, which presumably account for the slight elevation of the absorption in the germinal vesicle in the region of the nucleic acid band. Thus the absorption data agree with the chemical data in showing that the major part of the nucleic acids of the sea urchin

egg belong to the ribonucleic acids. (Only the pentose nature of the carbohydrate has actually been determined for the sea urchin nucleic acid;[9] but all of the nucleic acids so far analyzed in detail are either ribose or desoxy ribose.) Moreover this ribonucleic acid is not only present in the nucleolus but its maximum cytoplasmic concentration is close to the nuclear membrane.

For the spinach cells similar measurements were made of nucleoli and also of cytoplasm for one of the cells (Fig. 2). The cells chosen for measurement were in early prophase, following a double reproduction. In these cells there is no striking accumulation of absorbing substances around the nuclear membrane. Both nucleolus and cytoplasm have their absorption maximum at 2600; the chief component may then be ascribed to the pyrimidine band of the nucleic acids. Comparison with the absorption spectrum of yeast nucleic acid in concentration sufficient to give the absorption of the cytoplasm at 2600 Å ($5.3 \times 10^{-7}$ mg./ml.) shows, however, that other absorbing substances, presumably as in the case of the sea urchin protein in nature, are present. Only the chromosomes give the Feulgen reaction; hence these nucleic acids in the nucleolus and cytoplasm of spinach are not desoxyribose acids. In these cells, presumably secretory in function, there is again the association of plentiful ribonucleic acid with active growth.

The nucleoli of the salivary gland in *Drosophila melanogaster* are Feulgen negative, and not so pronouncedly basophilic as the two cases previously discussed. They are typical "plasmosomes," with considerable variation in their cytological appearance, stainability, etc. The absorption spectra of the cytoplasm in the salivary gland will be discussed elsewhere; they vary with the stage of development of the gland, beginning with a high nucleic acid concentration, which decreases as the gland matures. Accumulations of absorbing material are sometimes seen around the nuclear membrane.

In figure 3, III, the absorption spectra are given of nucleoli from a single gland of a male larva from the *Swedish B* stock (one of the standard "wild" stocks). The curves belong to the same family, the differences being minor ones in height, due possibly in some cases to variation in thickness after smearing, as well as to slight differences in composition. They all show a high absorption at the short wave-lengths, a hump around the nucleic acid maximum, and another lower level around the tyrosine-tryptophane band of the proteins. This nucleolus contains therefore a nucleoprotein with a lower percentage of nucleic acid than the others tested, so that the absorption of the proteins themselves plays a dominant rôle in the determination of the characteristics of the curve.

The absorption spectra of nucleoli in two sister females were also measured (Fig. 3, IV). In these nucleoli the nucleic acid maximum is still

further masked; the curve can be fitted by that of a nucleoprotein containing about 3.5% nucleic acid, the protein having the absorption characteristics of serum albumin. The curve for the male necessitates a different type of protein and about twice the percentage of nucleic acid. It is indicated, therefore, that the composition of the nucleoli is not a constant character but may vary within the species according to the genetic composition of the individual. This problem will be further discussed in the succeeding paper.

*Discussion.*—These three cases are, we believe, representative of various types of nucleoli. "True" nucleoli take basic dyes, are variable from group to group and give a negative Feulgen reaction (except for a few isolated cases[9] and for the so-called chromatic inclusions which are of interest in the problem of nucleolar synthesis). These characteristics, as well as their diverse behavior in digestion with enzymes,[10] are adequately explained by the assumption that the nucleoli are composed of ribonucleoproteins, containing varying percentages of nucleic acid. Indeed, since this work was completed Brachet[11] has shown that the ribonuclease of Dubos deprives Amphibian nucleoli and cytoplasm of their capacity to take the pyronin of the *Unna* methyl green-pyronin mixture, which would indicate that these nucleoli and cytoplasms also contain ribonucleic acids which are responsible for their basophily, in agreement with our view. The "specific" nucleolar stain of Semmens and Bhaduri[12] gives evidence of another sort. The technique involves the Feulgen procedure, after which the tissue is left in 5% sodium carbonate solution for an hour, to be stained in fast green thereafter and differentiated in 70% alcohol saturated with the carbonate. Under these conditions the fast green stain remains in the nucleoli. It seems likely that the ribonucleic acids are dissolved in the carbonate solution, since a similar procedure can be used for their extraction when it is desired to separate them from the thymonucleic acids (Delaporte).[13] The reaction to the stain after the solution of the ribonucleic acids would then be due to the presence of highly basic proteins (protamines, histones) in the nucleolus. There are no detailed experiments on the reactions of ribonucleoproteins with dyes, such as those carried out on the thymonucleic acids.[14] It seems likely that the variations in the basophily of the nucleoli may be explained by the old hypothesis of variation in the nucleic acid content. Parenthetically, it may be remarked that claims of the presence of lipoids in the nucleolus are based mainly on the destruction of the structure by "lipoid solvents." Since these are also protein denaturing agents, the use of such methods is not instructive. There is to date no good evidence of lipoids in the nucleolus.

The rôle of the nucleic acid compounds, particularly the nucleoproteins, in the general metabolism of the cell occupies increasing prominence, above all in the processes of synthesis accompanying growth and secretion

The enzymatic rôle of nucleoproteins in biological oxidations has been shown by the work of Warburg[15] and others; the viruses as they are purified and analyzed one by one fall consistently into the same group;[16] and cases are gathering in which the nucleoproteins act as growth factors. The cytochemical approach traces the correlation between the occurrence of changes in the composition of the parts of the cell itself and the processes going on at the time. We have already reported for a number of cases that cells in which active synthesis or growth is occurring are rich in the cytoplasmic ribonucleic acid compounds.[5] The present data permit

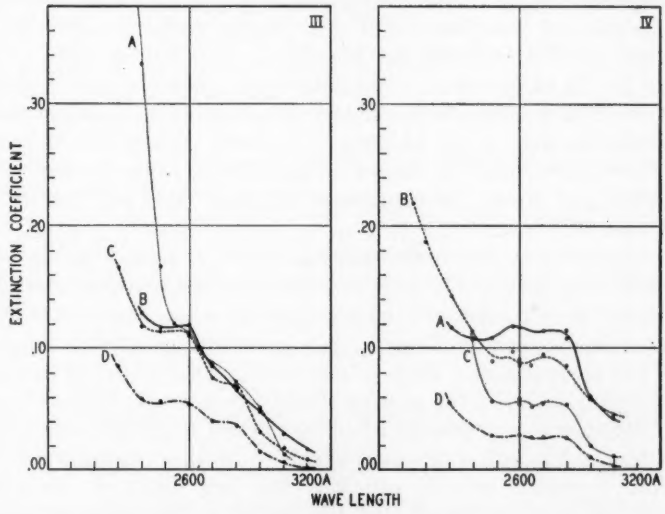


FIGURE 3

Ultra-violet absorption spectra of male (III) and female (IV) nucleoli from the Swedish B stock raised at 25 degrees. The male nucleoli are all from the same larva; among the females, *A*, *B* and *C* are from the same individual, *D* from another. Optical conditions: objective 2.5, ocular 10 ×.

the view to be discussed that these substances have their origin in the synthetic activity of the nucleus.

In the sea urchin eggs the nucleolus has a high content of ribonucleic acid compounds, the nuclear sap a low content, and there is an accumulation of these substances in the cytoplasm around the nuclear membrane. There is thus no evidence for a direct transport of these substances from the nucleolus to the cytoplasm before a breakdown of the germinal vesicle. But they already are present in the cytoplasm at this time; it would seem, therefore, from the presence of a gradient from the nuclear membrane peripherally that the synthesis occurs at that locus. In this way the syn-

thesis of nuclear products influencing cytoplasmic activity would take place at a surface which by its very nature is the joint product of both.

It should be noted that such a gradient around the nuclear membrane will not necessarily be apparent unless the rate of synthesis exceeds the rate of transport. Thus such accumulations of absorbing material around the nuclear membrane do not occur noticeably in the root tip of either *Allium* or *Spinacia*. Nor do they occur around cleavage nuclei. But according to Brachet[9] the ribose nucleic acids are depleted during the early development of various marine eggs, while the thymonucleic acid content rises correspondingly. Thus during the cleavages in which very rapid division proceeds the ribose nucleic acid content decreases, and it would seem that here the cytoplasm may somehow serve as a direct source of precursors for the chromosomes. The data do not, however, support Painter's[17] view that the chromosomes endomitotically multiplied during oögenesis themselves serve to provide these cytoplasmic precursors. As has already been shown here for the sea urchin, and the same is true both in *Drosophila* and *Musca*, the cytoplasmic synthesis takes place before the breakdown of the nuclei.

The rôle of the nucleoli in the synthesis of the cytoplasmic nucleic acids is difficult to evaluate. The generalizations about nucleoli that were made very early[18] are still applicable and are suggestive when considered in terms of the ribonucleic acid synthesis. Nucleoli of cleavage cells are in general small and inconspicuous; nucleoli from cells in which active synthesis is proceeding (gland cells, the growing oöcyte) are generally large and complex; disappearance or breakdown of the nucleoli in mitosis is correlated with the development of the chromosomes. In some cases extrusion of nucleolar fragments into the cytoplasm has been described. In recent years it has been established that the nucleoli are produced at specific chromosomal loci in heterochromatin.[19] The various data can be accommodated under the view that these chromosome regions are especially concerned with the ribonucleic acid metabolism of the cell. Evidence for this view is given in the succeeding paper. Here we need only say that the activity of the nucleoli is closely associated with an intense synthesis of the cytoplasmic ribonucleic acids. And, as has already been pointed out, the association of these substances with the processes of synthesis seems well enough established so that their study from the point of view of the nucleocytoplasmic relationships may provide some insight into the mode of action of the genes.

* Carnegie Institution of Washington; during the time this work was done, International Fellow of the Rockefeller Foundation.

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## *THE GENETIC CONTROL OF NUCLEOLAR COMPOSITION*

BY JACK SCHULTZ,* T. CASPERSSON AND L. AQUILONIUS

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY, AND KEMISKA INSTITUTIONEN, KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN

Communicated July 13, 1940

In a sense the study of the nucleolus occupies a key position in the analysis of the inter-relations of the nucleus and the cytoplasm. It is a structure within the nucleus, resembling the cytoplasmic structures in the type of nucleic acid it contains; at the same time, like other genetic effects, it is related to a definite locus in the chromosomes. Moreover, as has already been discussed in the previous paper, its function appears somehow to be connected with the synthetic activities of the cytoplasm. This being the case, the effects of genetic changes in the nucleolar regions upon the composition of the nucleolus should give some insight into the possible uses of this structure for the analysis of the problems associated with the action of the genes. Viewed from another aspect, changes in the composi-

tion of the nucleolus due to changes in the chromosomes should allow an additional attack on the relation of the thymonucleic and the ribonucleic acids. Evidence is here presented that the characteristics of the nucleolar nucleoproteins in *Drosophila melanogaster* are subject to change by genetic factors.

The nucleolus in *Drosophila melanogaster* has been shown to be associated with the heterochromatin of the *X* and the *Y* chromosomes.[1] A more detailed analysis of the available data gathered by different workers on its locus within the heterochromatic regions in the salivary gland chromosomes indicates that the locus of the nucleolar region in the *X* heterochromatin is close to, if not identical with, that of the locus for the large block of thymonucleoprotein in the metaphase chromosome.[2] Whether the nucleolar region of the *Y* is identical with that of the *X* cannot be determined at present. In the preceding paper we have shown the existence of a sexual difference in the nucleolar composition; this difference may be due to a difference between *X* and *Y*, the male containing both an *X* and a *Y* chromosome, the female containing two *X*'s. In the following, measurements of the absorption spectra of the nucleoli of males and females containing various chromosome rearrangements are described. The data are exploratory in nature, and having been collected upon preparations used for other purposes as well do not form as simple and complete a series as could have been planned for the special study of the nucleolus.

Two *X* chromosome rearrangements were used, one of which, symbolized as $X^c$,[3] involves a break in heterochromatin within the nucleolar region. The other rearrangement, $X^{c^2}$, also has one break in the heterochromatic region, but just outside the nucleolar region. Both of these are so-called "closed" or ring chromosomes. They are the result of translocation between the members of a pair of attached *X* chromosomes,[4] being deficient for a very small part of the tip of the *X* chromosome. Each contains a duplication of the heterochromatin from the point of rearrangement to the spindle attachment region. Thus for $X^c$ the duplication extends from the nucleolar region to the spindle attachment; but $X^{c^2}$ contains a duplication for all of the *X* heterochromatin. Hence the studies of these chromosomes involve simultaneously the effects of rearrangement and of duplication. For the *Y* chromosome, the rearrangement studied is of a simpler nature, being an intercalation into the *Y* of a part of the second chromosome.[5] In an attempt to test the effect of heterochromatin other than that around the nucleolar regions, measurements were made of a rearrangement involving the exchange of part of the *X* chromosome for most of the small fourth chromosome, with the point of rearrangement in chromosome four being in heterochromatin, and that in *X* at the section of Bridges' map $3F_1$.[6] The technique applied has already been described in the previous paper.

The absorption curves for the male nucleoli are shown in figures 1*A–E*, II*D* and IV*D–E*. Those for the *Swedish B* (the "wild type" stock)

male have already been presented in the preceding paper. In each case where duplicate measurements are available they are of different nucleoli from the same individual. It is evident that the different types give

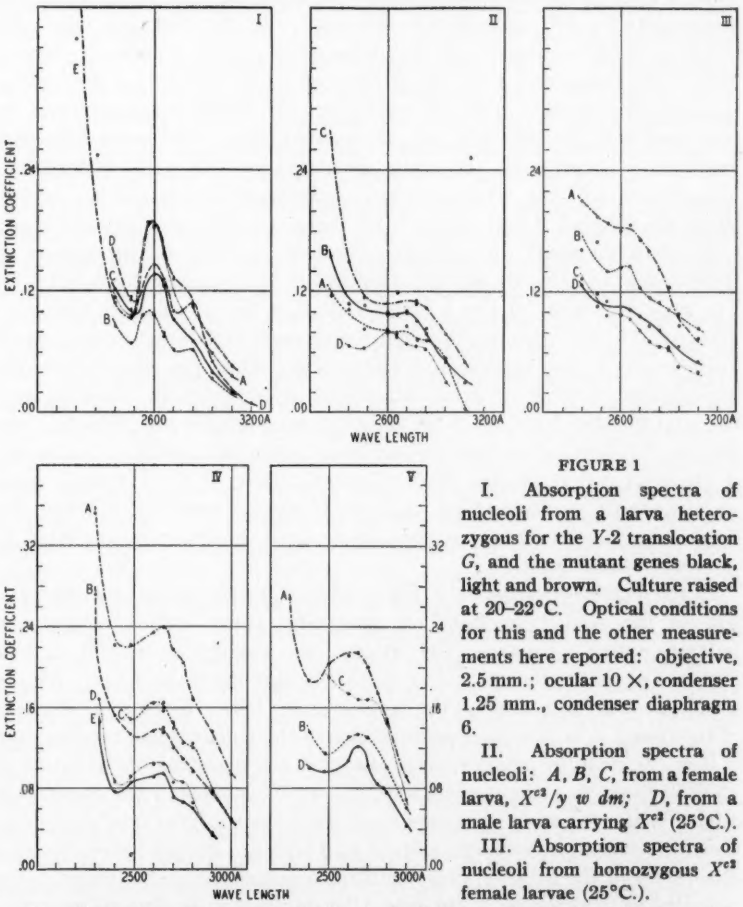


FIGURE 1

I. Absorption spectra of nucleoli from a larva heterozygous for the *Y*-2 translocation *G*, and the mutant genes black, light and brown. Culture raised at 20–22°C. Optical conditions for this and the other measurements here reported: objective, 2.5 mm.; ocular 10 ×, condenser 1.25 mm., condenser diaphragm 6.

II. Absorption spectra of nucleoli: *A*, *B*, *C*, from a female larva, $X^{c2}/y\ w\ dm$; *D*, from a male larva carrying $X^{c2}$ (25°C.).

III. Absorption spectra of nucleoli from homozygous $X^{c2}$ female larvae (25°C.).

IV. Absorption spectra of nucleoli: *A*, *B*, *C*, from a female larva heterozygous for $w^{vD3}/y\ X^{c}$ (raised at 16°C.); *D*, *E*, from a male carrying $y\ X^{c}$ raised at 25°C.

V. Absorption spectra of nucleoli: *A*, *B*, from a female larva $w^{vD3}/y\ w\ dm$ raised at 18°C.; *C*, the same at 25°C.; *D*, from a female larva $w^{vD3}/y\ w\ dm$; *Y* raised at 18°C.

characteristically different groups of curves, where the data are adequate to permit a judgment. The nucleoli of the Y-2 translocation male have absorption spectra that show a maximum around 2600 A, with a hump at a

lower level corresponding to the band around 2800 of tyrosine and tryptophane. The males containing the closed $X$ chromosomes show a different type of curve, with the 2600 peak more submerged in the "protein" absorption. A distinction between the two closed $X$'s is evident, although the scanty data for $X^{c2}$ make caution necessary. In the wild type male, the 2600 maximum is present only as a hump in the curve, similar to that at 2800. The differences might be interpreted as due either to a decrease in the amount of protein in the different types containing rearrangements, as compared with the wild type, or, conversely, there could be an increased amount of nucleic acid. In order to determine this point adequately, measurements of the total amount of substance are necessary. As between the *Swedish B* and the $T(Y\text{-}2)G$ males, the difference lies in the absorption at the short wave-lengths, since the ratios of the absorption at 2600 to that at 2800 are sensibly identical in the two cases (1.6 and 1.7). The ratio is similar for the $X^c$ male (1.5), but the $X^{c2}$ male is distinctly lower (1.3). It is thus possible that the increase in heterochromatin gives an increase of both nucleic acid and protein, while the effect of the rearrangement is to decrease the amount of protein formed. In such case the intermediate character of the $X^c$ curve as compared with the wild type and the $Y$-translocation curves would be the result of a position effect on the $X$ nucleolar region plus the effect of a duplication; in the $Y$-translocation we observe the position effect itself and in the $X^{c2}$ male the effect of the duplication itself. These interpretations must, however, be regarded as provisional at present.

In the female series the data (Fig. 1, II ($A$–$C$); III) permit the comparison of the wild type from the preceding paper and $X^{c2}$ with the heterozygous $X^{c2}$ female. The ratios of the absorptions at 2600 and at 2800 are again instructive. For the $X^{c2}$ female the ratio is 1.28; for the wild type it is 1.07; and for the heterozygote, 1.08. Evidently the effect of the closed $X$ in heterozygous condition in the female is less marked than it is in the male, although even in this case a comparison of the ratios at 2650/2800 shows a slight effect: $X^{c2}$, 1.29; $X^{c2}/ywdm$, 1.15; *Swedish B*, 1.03. Without a more complete analysis of these curves into their component absorbing substances, and, as said above, a knowledge of the total absorption of the nucleoli, only tentative conclusions can be drawn. Apparently in this case as in the male, the duplication in the $X^{c2}$ increases both the nucleic acid and the protein absorption. The question of the dominance of the normal nucleolar composition in the heterozygote where two differently produced nucleoli are fused is more complex. It brings up again the difference between the nucleolar composition of the sexes. As previously pointed out, this difference may be the consequence of the presence of the $Y$ chromosome in the male, or it may be the result of the general difference of genic balance as between the sexes. A sexual differ-

ence similar to that found in the *Swedish B* measurements is found in the male and female $X^{c2}$, with the protein absorption again more marked in the female. If the effect of the $X^{c2}$ be assumed to be due to a duplication of heterochromatin including the nucleolar region, the results fall into line with the behavior of duplications for $X$ chromosome regions in *Drosophila* in general: the effect, that is, the difference from the wild type, is greater in the male than in the female for a duplication of any given part of the $X$. It is of interest that the complicated relations of genic balance should be met with in intra-nuclear characters; McClintock has already described a similar case in the behavior of the nucleolar region of *Zea mays*.

For the analysis of such possible genic balance relations, study of the effects of heterochromatic regions other than the nucleolar region itself should be of value. To this end measurements were made of the nucleoli in individuals heterozygous for the $X$-4 translocation (symbolized $w^{sD3}$) previously referred to, and for the same chromosome as that used in the heterozygote for $X^{c2}$, containing the mutant genes yellow, white and diminutive; a measurement was also made of a similar heterozygote containing an extra $Y$ chromosome. The data (Fig. 1, V) give a curve of marked nucleic acid character, with an evident effect of the extra $Y$ chromosome in accentuating the maximum at 2600. These preparations, however, were made (in connection with studies of the variegated types correlated with rearrangements of this kind) from larvae raised at 18 degrees. A check measurement of a control raised at 25 degrees, and therefore directly comparable to the closed $X$ heterozygote, shows a flatter curve. The absorption ratio for 2600/2800 is only 1.19, as compared with the ratio for the 18-degree series of 1.37. It appears, thus, that there is an increase in the ratio of nucleic acid to protein at the lower temperatures, perhaps similar in nature to the effects of rearrangement in the nucleolar regions. There seems still, however, to be an effect of the $X$-4 rearrangement on nucleolar composition; for even at 25 degrees the 2600/2800 ratio (1.19) is higher than the value of 1.08 found in the $X^{c2}/y\ w\ dm$ heterozygote. The rearrangement here discussed also has the effect of inducing variegation for characters due to genes located in the regions transposed to heterochromatin; these changes have been postulated on the basis of changes seen in the salivary gland chromosomes to result from changes in the nucleoprotein ratio of the genes themselves. Should the present indication of an effect on the composition of the nucleolus be substantiated, evidence can be obtained for the interrelations of nucleolar and chromosomal composition. It would follow that in addition to the chromosomal effects of these rearrangements there is also an effect of heterochromatin on the nucleoprotein metabolism of the nucleolus.

The effect of the $X$-4 translocation on the nucleolus must be regarded as dominant, since it is manifested in the heterozygous female. It contrasts

with the effect of $X^{c2}$ in this regard. We took the occasion to make measurements of heterozygotes for the *X-4* rearrangement and for $X^c$; it seemed possible that the more pronounced departure of $X^c$ from the normal in the male might show in the heterozygous female as well. These heterozygotes

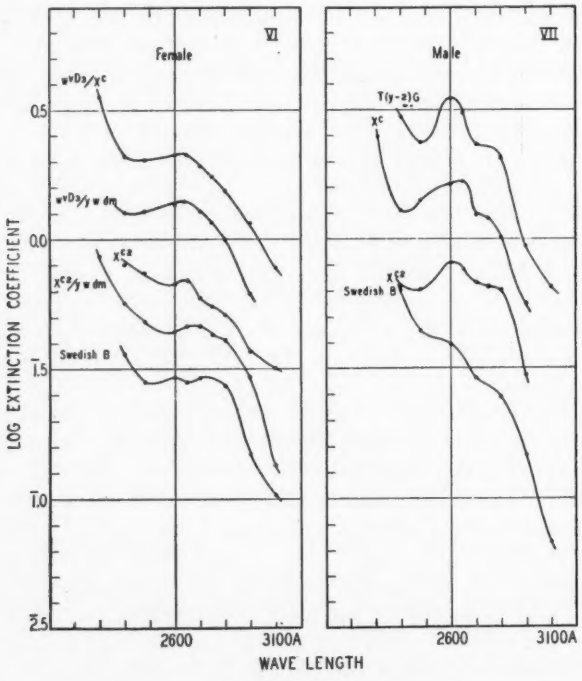


FIGURE 2

The data of the previous figures are summed for each type, and the logarithms of the extinction coefficient are plotted. For purposes of clarity they are placed at convenient distances on the scale of ordinates. The data for the *Swedish B* males and females have been summed, and the logarithms of the sums plotted directly. To the logarithms of the other sums the following have been added before plotting: females, $X^{c2}/y\ w\ dm$, 0.6; $X^{c2}$, 0.1; $w^{vD3}/y\ X^c$, 0.6; males, $X^{c2}$, 1.0; $X^c$, 0.9; $T(Y\text{-}2)\ G$, 0.8.

were raised at 16 degrees instead of 18, as were the other heterozygotes for this rearrangement. But in any case, as the 2600/2800 absorption ratios calculated from the data (Fig. 1, IV, *A*, *B* and *C*) show, there is no striking difference from the $w^{pd3}/y\ w\ dm$ heterozygote. In the former case they are 1.41, 1.46 and 1.32, as compared with 1.42, 1.31 in the latter case.

*Discussion.*—A comparison of the different sets of data is made in figure 2. For each type the logarithms of the sums of the extinction coefficients of the individual nucleoli are plotted against the wave-length. In this way curves are obtained whose shape is independent of variations in concentration or thickness of the absorbing layer.[7] They are dependent solely on the nature of the constituent absorbing substances. Thus the logarithmic plots of the sums of the extinction coefficients for all the nucleoli of a given type give an estimate of the nucleolar composition such as would be obtained by an analysis of the composition of a mixture of all the nucleoli for each type. The different types can then be compared since nucleoli of like composition should give curves which are superposable each upon the other. It is evident that this is not the case for any of the types so plotted. The relations previously discussed emerge with especial clarity; the nucleic acid protein ratio is increased in the types with the rearrangements, as the height of the 2600 band shows. The males have in general a more pronounced maximum in the cases where direct comparison can be made. The females have a gentler group of curves, in which the protein absorption is more influential. But each of the genotypes has its own characteristic curve.

We have discussed the differences in the absorption curves as due to genetic differences. The objection might be raised that within an individual the data are consistent, but that random non-genetic differences between individuals could account for the differences observed. This seems unlikely, since the seriation of the types is self-consistent, and the agreement between such types as the $X^{c2}/y\ w\ dm$ and the *Swedish B* female is so close as to permit the discussion of dominance even though the stocks are unrelated. Also, as is discussed below, the staining reactions of the nucleoli run parallel to our measurements. It follows then that the analysis of these changes in nucleolar composition is an analysis of the function of the genes in the formation and behavior of the nucleolus.

The "nucleolar genes" are located in the heterochromatic regions, and they must therefore partake of the properties of those regions, if indeed they are not responsible for some of them. Heterochromatic regions have the capacity (1) to form large amounts of thymonucleic acid (or, better perhaps, thymonucleoprotein) in the chromosomes themselves; (2) to form[8] or affect the composition of the nucleoli; (3) to affect the characteristics of neighboring regions translocated to them in such a way as to change the developmental effects of these regions in somatic cells[9,10]; (4) to affect the content of the ribonucleic acids in the egg cytoplasm of *Drosophila*.[6] Only the first and second of these characteristics are known for organisms other than *Drosophila melanogaster*, and they appear to be quite general properties. We are not here concerned with the specific cytological characteristics such as the property of indiscriminate synapsis, and the

type of chromomere formed by these regions in the giant chromosomes.

It would be premature to attempt a detailed correlation of these properties. In outline, however, they seem all to be different aspects of the nucleic acid metabolism of the cell. For the nucleolus, the evidence here given permits the further specification that the ribonucleoprotein metabolism is controlled by these regions. Thus the connection becomes close between a region of the chromosome which produces much thymonucleic acid and a structure composed of ribonucleoprotein. How direct this relation is cannot be told, for in *Zea mays*[9] the smaller portion of the nucleolar region forms the larger nucleolus and the presence of an additional nucleolar region has no effect on nucleolar size. On the other hand in *Solanum*, M. M. Lesley[11] has shown a quantitative correspondence between the size of the nucleolus and the size of the nucleolar region, in a series of variants. Failing measurements of the composition of these nucleoli to show that the differences are merely quantitative, definite statements are precarious. It seems likely, however, that the difference of behavior between *Zea* and *Solanum* is another example of the difficulties attendant on the use of the effects of deficiency or duplication of a chromosome section in the analysis of the effects of that section. For the composition and size of the nucleolus are dependent on the general genic balance, and only when the nucleolar region itself is the limiting factor in nucleolar production can we expect to see changes in the characteristics of the nucleolus due to it. Indeed McClintock has shown in *Zea* that other chromosome regions very easily take over the rôle of nucleolar synthesis; it is to be questioned whether this would so easily occur in *Solanum*.

There are wide variations in the behavior and the staining reactions of the nucleoli of even closely related species. The present results are particularly relevant to the variations described by Bauer[12] in the Chironomids in which some species show in aceto-carmine preparations a well-organized deeply staining nucleolus, others a light and diffuse type. In the present series in *Drosophila* similar variations are encountered, and indeed were the origin of this investigation. The parallel between the intensity of stain, the compactness of the nucleolus and the prominence of the nucleic acid band is rather good; in other words, the structural qualities of the nucleoli seem to be associated with their nucleic acid content. This indicates that the ribonucleic acids as well as the thymonucleic acids have a rôle in structure formation of the sort emphasized by Hammarsten.[13] In this way the genotypic control of the structural characteristics of the nucleolus may be mediated by changes in its nucleic acid content.

The heterochromatic regions are commonly known as genetically "inert" because where tests have been made no phenotypic changes are detected in the presence of duplications or deficiencies. It is evident from the data reported and discussed here and elsewhere that the "inert" char-

acter is factitious. Actually the situation is that of a system in which all components are present in excess, similar to that discussed in connection with the nucleolar region in *Zea*. This is not surprising, if as we have suggested in the previous paper the ribonucleic acid compounds are influential in a variety of cellular syntheses. On this view the heterochromatic regions are particularly concerned with the synthesis of the nucleic acids; in the chromosomes their activity is manifest in the blocks of thymonucleoprotein they produce; in the nucleolus and the cytoplasm they produce the ribonucleic acid compounds. In the formation and development of the nucleolus it is possible that we have, so to speak, a pattern of gene function; the ribonucleoprotein is formed at telophase, and in endomitotic nuclei grows parallel with the growth of the chromosomes. It remains to discover how far the specific effects of genes in development are part of the same or a similar system of nucleoprotein metabolism; the rôle of the heterochromatic regions in variegation suggests that such a possibility is not unlikely.[14]

* Carnegie Institution of Washington; during this work, International Fellow of the Rockefeller Foundation.

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[14] The data reported in this and the preceding communication were presented in part in papers read at the Seventh International Genetics Congress (1939). The work has been supported by funds from the Rockefeller Foundation and from the Stiftelsen Thérèse och Johan Anderssons Minne.

# ARE DIFFERENCES IN SIZE BETWEEN PARTS OF THE BODY DUE TO GENERAL OR SPECIFIC FACTORS?

By J. I. Cohen

Psychological Laboratory, University College, London, England

Communicated June 12, 1940

The dispute between Castle[1] and Sumner[2] on the problem expressed in the above title may perhaps be indirectly resolved by results obtained by the writer in a different connection. Castle maintains that the genetic influences determining total size in rabbits are general ones affecting all parts of the skeleton simultaneously, while special factors (if any) limiting the size of particular bones play only a subordinate rôle.[3] Sumner, however, believes that size factors specific to certain limbs and parts of the body are more important than general factors in determining body size as a whole. Davenport holds what appears to be an intermediate view, namely, that general factors control growth "only to a degree that may be estimated as less than a half." Shull supports Castle's contention.

The present writer, like Davenport, has been working on measurements of man, not of rabbits, and has not been primarily concerned with the problem of the *inheritance* of human size.[4] Nevertheless, the observed results obtained by him seem to admit of a genetic interpretation.

Briefly, a large number of anthropometric measurements of several groups of adult persons were obtained. These measurements were then intercorrelated and analyzed by means of the factorial techniques currently used in statistical psychology. The object of the analysis was to extract a minimum number of factors accounting for a maximum amount of the total variance of the traits. The measurements include head length, head breadth, head circumference, head "diagonal," trunk length, chest breadth, chest depth, chest circumference, stature, sitting-height, arm length, leg length, shoulder breadth, pelvic breadth, pelvic circumference, waist circumference and various facial measurements.

The samples consisted of 164 male and 106 female adult psychotics and of 50 normal adult males.

In numerous analyses of the measurements of the subgroups, it was found that the bodily parts could best be regarded as determined by two major growth reactions, one governing magnitude in all physical dimensions and the other governing disproportionate growth in length measurements on the one hand, or in circumferential measurements on the other. In other words, we may consider individual differences in the external measurements of the body to be determined by a process making for general magnitude and a process making for proportions of the parts. The variance of the former is much greater than that of the latter.[5]

In order to get a measure of a person's mass or bulk in the linear sense, corresponding to a ponderal measure or to the measure of the surface area of the body, it is necessary to obtain a weighted mean of representative measurements of the body in all its three dimensions. This can be provided by the first factor saturations in the traits which are measured. Now even when we know a person's bulk, we do not know his shape or proportions. We do not know whether he has relatively long limbs and short trunk or vice versa, we do not know his head shape and so on. This information can be supplied by weighting the initial measurements with saturations of the traits in the second factor.

The amount of variance accounted for by the first and second factors varied in the different subgroups as may be seen below:

| | PERCENTAGE OF TOTAL VARIANCE ACCOUNTED FOR: BY FIRST FACTOR | BY SECOND FACTOR | |
|---|---|---|---|
| $N$ = 64 males | 31.9 | 12.2 | 14 traits analyzed |
| $N$ = 50 " | 43.6 | 24.1 | 14 " " |
| $N$ = 62 females | 34.4 | 12.5 | 12 " " |
| $N$ = 86 males | 42.1 | 16.8 | 8 " " |
| $N$ = 86 " | 35.0 | 14.6 | 6 " " |
| $N$ = 62 females | 37.2 | 14.1 | 8 " " |
| $N$ = 33 " | 29.7 | 9.9 | 8 " " |

We may hazard the hypothesis that there are genetic influences determining growth or size of the body in all its dimensions, vertical, horizontal and sagittal. Over and above this tendency to grow to a certain bulk or magnitude, we may postulate the existence of genetic factors making for excess or differential growth longitudinally on the one hand, or circumferentially on the other.

If this hypothesis is accepted, the views of Castle, Sumner and Davenport must all be modified. General factors, in our analysis, appear to account for between twice and three times the amount of variance as specific factors. Davenport and Sumner would both seem to be underestimating the significance of general factors while Castle seems to overestimate them.

The view to which we are led here conforms well with that put forward by Pearl in his discussion of individual differences in bodily habitus. The following statement of Pearl sums up the position well: "The vertebrate plan of structure comprises a principal and primary bodily axis which is the longitudinal one, with cephalic and caudal ends. The secondary axes are two in number and at right angles to each other, the dorso-ventral axis and the lateral axis. The external form—somatology or bodily habitus—of such an animal is plainly bound in general to depend upon the relative or proportional growth along each of these axes."[6] I would like to add that growth in bulk proceeds in all three dimensions whereas growth in shape or

proportions proceeds differentially in the first *or* in the second and third dimensions.

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[2] Sumner, F. B., *Ibid.*, **10**, 178–180 (1924).
[3] Castle, W. E., *Carnegie Inst. Publ.*, No. 320 (1922).
[4] Cohen, J. I., *Nature*, **144** 944 (1939).
[5] Cohen, J. I., *J. Mental Science*, **86**, No. 362 (1940).
[6] Pearl, R., *Constitution and Health*, London, Kegan Paul (1933).

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## *A PENTAPLOID LARVA OF THE NEWT, TRITURUS VIRIDESCENS*

BY GERHARD FANKHAUSER

DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY

Communicated July 11, 1940

During the past two years a search has been made for exceptional polyploid individuals among salamander larvae, by means of chromosome counts in amputated tailtips. Several triploid larvae have been found in the common newt, *Triturus viridescens*,[1] and triploid as well as tetraploid larvae in the two-lined salamander, *Eurycea bislineata*.[2]

In November and December, 1939, about forty larvae of *Triturus viridescens* were raised by the students in a course on animal development, from eggs which had been obtained by pituitary implants. On December 15 the larvae were turned over to Miss Rita Crotta for fixation. While examining the animals she noticed a suspicious pigment pattern in one larva which had larger but fewer melanophores than the rest. This type of pigment pattern had been found before to be a reliable indication of polyploidy.[2] Moreover, the pattern seemed to be sufficiently different from that previously observed in triploid larvae to indicate a higher degree of polyploidy, probably tetraploidy.

The tailtip of this larva was at once amputated, fixed and stained *in toto*. The nuclei of the various tissues were much larger than in the diploid or triploid tailtips. Unfortunately, the epidermis of the tailfin, in which the chromosomes can be most easily counted, contained only very few mitotic figures, and none of these were in metaphase.

Within two weeks the larva had regenerated a small new tailtip. This was amputated together with a piece of the original tail. This second tailtip preparation contained one good metaphase in the regenerated portion. In this large and crowded figure fifty-four chromosomes could be counted, a number which is as close to the pentaploid, fifty-five, as one could expect considering the difficulties of the enumeration.

A week later the whole animal was fixed, and the posterior end of the tail, with a small regenerated cone, was stained and mounted. In this third tailtip preparation one fairly good metaphase was discovered which contained certainly more than forty-eight chromosomes.

While both counts indicated that the larva was more than tetraploid, probably pentaploid, the evidence was not considered sufficient. An extensive series of measurements of nuclei was planned with the expectation that a comparison of the size of the nuclei with that in diploid and triploid larvae would contribute reliable evidence of the chromosome number. However, when the whole animal was sectioned later on, a considerable number of mitoses were found in the central nervous system and the intestine. In the former, the figures are too small and crowded to allow even an approximate count. In the intestine, the cells are larger, and the chromosomes more widely spaced. Still there is too much overlapping of the chromosomes in intact metaphase plates which are complete within a single section and normally considered to be the most reliable figures for chromosome counts. However, several metaphase plates had been divided by the knife in such a way that few chromosomes were broken, and a count of the intact elements in the two adjacent sections was possible. Since all the chromosomes of *Triturus viridescens* are V- or J-shaped, fragments of chromosomes not containing the spindle attachment point are quite easily recognized. In six metaphases from various regions of the intestine the following chromosome numbers were counted:

54 (very few fragments present),
53 ( " " " " ),
at least 52,
" " 51,
" " 50,
" " 50.

These numbers include only those chromosomes that could be made out clearly. Moreover, one or more chromosomes may have been missed if they were cut at or near the spindle attachment point and were therefore recorded as fragments. It is thus highly probable that the actual number in all these figures is pentaploid, fifty-five.

A high degree of polyploidy is also indicated by the very large size of the individual nuclei in the various tissues of the tailtip (Figs. 2 to 7). For instance, the nuclei of the epidermis cells, which are flat discs, appear more than twice as large as those of the diploid larva (Fig. 2). When twenty nuclei of each animal were drawn on cardboard and weighed, the ratio of the weights was found to be close to five to two. The cell boundaries are not clearly visible in tailtip preparations except in the gland cells in the epidermis (Fig. 7) which show that the cell size has increased in proportion

to the size of the nucleus. This is also demonstrated by the increase in size of the melanophores over the whole body of the animal (Figs. 8 and 9).

Since rather complete records of the development of all eggs had been kept by the students in the course, it was possible to trace the development of the pentaploid back to the gastrula stage. As far as the records show, the early development of the pentaploid embryo proceeded normally and at the same rate as that of the diploid embryos. When the pentaploid came under closer observation as a young larva, at the age of three weeks, it did not differ markedly from the diploid controls, with the exception of the pigment pattern. A photograph taken a few days later shows that the pentaploid was only slightly larger than the control (Fig. 8). The absence of pronounced gigantism agrees well with the observations on triploid and tetraploid salamander larvae reported previously.[1, 2, 3] At all levels of

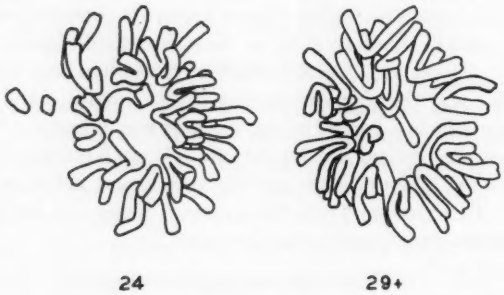



FIGURE 1

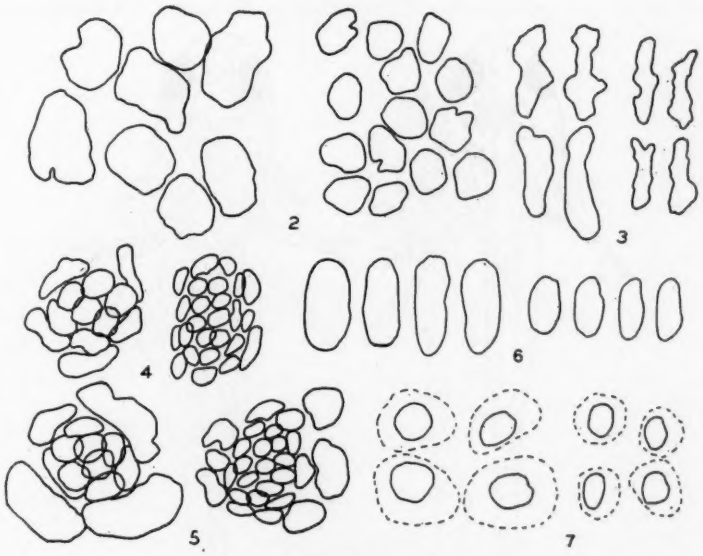
Metaphase plate from intestine containing at least 53 chromosomes in two sections. Camera lucida drawing. × 1533.

polyploidy, the increase in cell size is compensated by a corresponding decrease in cell number. This is well shown by a comparison of pentaploid and diploid lateral line organs in the tailtip preparations (Figs. 4 and 5).

The pentaploid began to feed on small Daphnia and pieces of Enchytraeus at the age of three and a half weeks, at the same time as the controls. The digestion of pieces of Enchytraeus seemed to proceed somewhat more slowly; it was observed twice that, twenty-four hours after a meal, some of the food was still in the stomach while in the controls it had all passed into the intestine. The rate of heart beat was normal; at the age of five weeks it was 76 per minute, as compared with 76 and 78 in two diploid larvae. The color of the heart, however, was light pink instead of deep red as in the controls, because of the smaller number and larger size of the erythrocytes. The difference in size and number of the red blood cells passing through the heart and gills was clearly visible under the binocular.

On several occasions it was noticed that the pentaploid reacted more slowly than the diploid larvae. Furthermore, from the age of four weeks on, fluid began to accumulate in the body cavity. The ascites gradually became more severe and extended to the heart region (Fig. 9).

On January 3, 1940, the pentaploid and a control were photographed and




FIGURES 2 TO 7

Camera lucida drawings of nuclei from different tissues of tailtip preparations. In each figure the pentaploid nuclei are on the left.

2. Epidermis of tailfin. × 270.
3. Connective tissue of tailfin. × 614.
4. Nuclei of corresponding lateral line organs on axis of original tailtip (No. 4 from end). × 313.
5. Nuclei of corresponding lateral line organs from third tailtip (last organ on dorsal fin). × 313.
6. Nuclei of erythrocytes, from capillaries of tailfin. × 614.
7. Nuclei and cell boundaries (broken lines) of gland cells (Leydig cells) in tailfin. × 270.

stayed in a solution of chloretone 1:5000 for over thirty minutes, a treatment which had never been found to have any harmful effect on diploid or triploid larvae. On the next morning the control was completely normal while the gills of the pentaploid showed the effects of an overdose of chloretone, i.e., an almost complete reduction of the small branches and a strong

curvature of the main stems. Because of its poor general condition the animal was preserved on the following day.

While the morphological effects of pentaploidy in this larva were thus rather slight, the high chromosome number seemed to have more serious physiological consequences, as is indicated by the early appearance of ascites, the reduced reactivity and the increased susceptibility to chloretone.

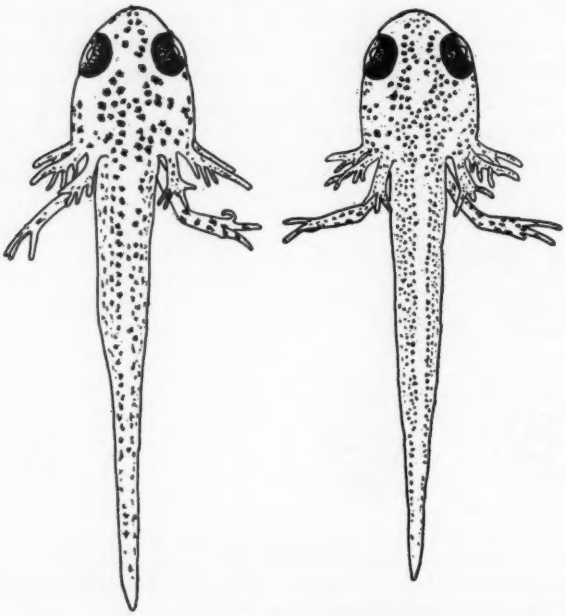



FIGURE 8

Pentaploid larva and diploid control, $3^1/_2$ weeks old. The pentaploid appears normal except for pigment pattern and slightly larger size. In both larvae the melanophores on head and body are "contracted." Tracings of photomicrographs. × 10.

It is possible that these phenomena were all connected with the great increase in cell size and the reduction in cell number in the various organs and tissues. The microscopical anatomy of the larva is under investigation at present and will be described elsewhere.

It is significant that the viability of the pentaploid salamander larva was much lower than that of the two tetraploid larvae of *Eurycea bislineata*

which were raised in 1939; these in turn were not as vigorous as the triploid larvae of both *Eurycea* and *Triturus viridescens*. Unfortunately, tetraploid larvae have not yet been found in *Triturus*, and attempts to induce tetraploidy by cold treatment of unsegmented eggs during the first cleavage mitosis have not been successful so far (unpublished observations of M. Perrot). There exists, therefore, still a gap in the polyploid series in this species which now extends from haploidy[4, 5] to pentaploidy.

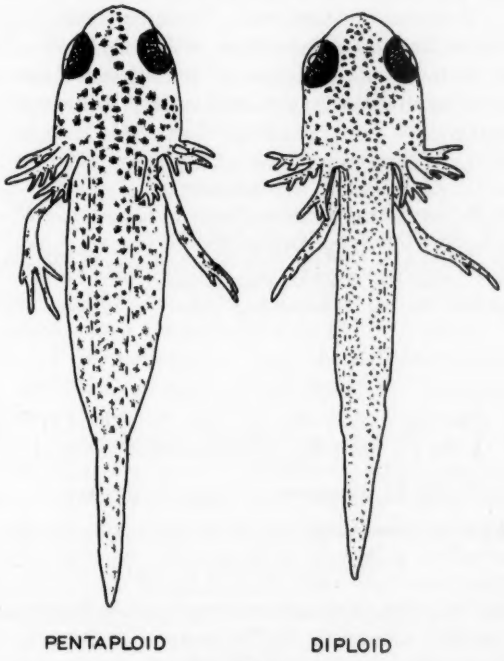



FIGURE 9

The same larvae as in figure 8, $5^1/_2$ weeks old. Body cavity of pentaploid distended with fluid (ascites). In both larvae the melanophores are "expanded." Tracings of photomicrographs. × 10.

No definite information is available concerning the origin of this exceptional pentaploid embryo. Triploid salamander larvae presumably arise in nature from the union of an exceptional diploid gamete with a normal haploid gamete. Tetraploid individuals would originate if two exceptional gametes should meet, or if a doubling of the chromosome number should take place during the first cleavage mitosis. Furthermore, additional sets

of chromosomes might be supplied by supernumerary spermatozoa, since the eggs of newts and other salamanders are normally polyspermic. Usually, the accessory sperm nuclei remain well separated from the pronuclei and degenerate before the first cleavage.[6] On rare occasions, however, two spermatozoa may possibly penetrate into the egg so near each other that the nuclei fuse, as is frequently seen in highly polyspermic eggs which do not cleave. In eggs of the salamander, *Hynobius retardatus*, which seem to be monospermic as a rule, Makino[7] has actually observed the fusion of three nuclei. However, whether such a situation would lead to a bipolar triploid mitosis or to a tetrapolar mitosis with irregular distribution of the chromosomes, as it does in dispermic sea urchin eggs, is not known, since the behavior of the division centers associated with the sperm nuclei has not been observed.

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## *UNEQUAL BREAKS IN TWO SISTER CHROMATIDS INDUCED BY X-RAYS IN DROSOPHILA MELANOGASTER*

BY M. DEMEREC AND EILEEN SUTTON

CARNEGIE INSTITUTION OF WASHINGTON, COLD SPRING HARBOR, N. Y.

Communicated July 13, 1940

A great deal of work, both experimental and observational, has been done on the number of strands per chromosome at different stages of the nuclear cycle, the time of splitting of the chromonemata and the effects of irradiation on split chromosomes.

From genetical experiments we have the results of Patterson[1] on mosaics derived from irradiating sperm of *Drosophila*, from which he concluded that the chromosomes were already split in some of the sperms (about one in seven) at the time of irradiation, but not in others. Moore,[2] who obtained similar results, supposed that the chromosomes of all the sperm were split at the time of breakage; mosaics would result from single chromonema breaks, but both chromonemata could be broken simultaneously at the same level, and sperm thus affected would give rise to flies with homogeneous mutant tissues.

Moore's conclusions are in keeping with the cytological data of Nebel[3]

and Sax.[4] These workers have shown by irradiation experiments on *Tradescantia* that a single x-ray "hit" can break either one or two chromonemata at a given point of a split chromosome.

Kaufmann[5] and Kaufmann and Bate[6] have published data on duplications in *Drosophila* salivary chromosomes. These studies show that one of two sister chromatids may be broken at one point, while both chromatids are broken at a second point in the same chromosome. In this paper a further example of differential breakage of two sister chromonemata will be described.

*Description and Results.*—The progenitor of *N*264-86 mutant is a single notch female found among the offspring of *y pn* females which were mated with x-rayed wild-type Swedish *B* males. Genetic tests showed that this notch is a dominant sex-linked character, lethal when homozygous and hemizygous, and that it belongs to the Notch series of changes localized in the 3.0 region of the X-chromosome.

The *N*264-86 females heterozygous for diminutive (*dm*) had diminutive bristles, and some of them showed mosaic spots covered with long, wild-type bristles. Since mottling is a characteristic feature of chromosomal rearrangements involving heterochromatin, this behavior of *dm* suggested that such rearrangement might have occurred in *N*264-86. Cytological studies confirmed this indication. They showed that a segment of the chromosome was taken out of the Notch region and inserted into heterochromatin of 4 at 101*F*.

Since the fourth chromosome segregates freely from the *X*-chromosome, the inserted piece also segregates independently of the *X*-chromosome from which it came. Consequently two recombination classes are found: (*a*) hypoploid class with the deficient *X*-chromosome but without the fourth carrying the insertion, and (*b*) hyperploid class with the insertion and the normal *X*-chromosome. The hypoploid class is viable and fertile in heterozygous females, and the hyperploid class is viable and fertile in both sexes.

*Analysis of Deficiency.*—Cytological analysis indicates that this deficiency involves the region from 3*C*8 to 3*E*5, inclusive (Fig. 1). In this study salivary glands were used from larvae which had one deficient and one normal *X*-chromosome. In a great majority of figures obtained with that material the bands present in both chromosomes show close synapsis. In order to confirm the analysis on unsynapsed chromosomes, slides were prepared from the salivary glands of larvae which had one deficient chromosome and the other carrying the translocation (1.4) 258-18, in which the tip of the *X*-chromosome following the band 3*C*4 is exchanged with 4. In many figures of that material *X*-chromosomes are unsynapsed at the distal end.

Genetic tests showed that the deficient chromosome carries the wild-

type allels of *w* and *ec*, and that it is either deficient or carries recessive allels of *rst*, *fa* and *dm* (Fig. 1).

*Analysis of the Inserted Segment.*—Cytological study shows that a segment from 3*C*7 to 3*E*5 is inserted into heterochromatin of 4 at 101*F*, in its normal position in relation to the centromere. By genetic tests it was determined that the loci *fa* and *dm* present in the segment show mottling (Fig. 1).

*Discussion.*—All adequately tested Notches behave genetically as deficiencies for *fa* and *spl*, and some in addition behave as deficiencies for the adjacent loci such as *w* and *dm*. Cytological studies of various Notches indicate that some do not show any cytologically detectable deficiency, while others are deficiencies for smaller or larger segments centering in the 3*C* region of the Bridges[7] salivary chromosome map. Since all cytological deficiencies include band number 3*C*7, that band is considered as representing the Notch locus. Similar evidence indicates that 3*C*1 represents the *w* locus, 3*C*4 *rst*, 3*D*1.2*dm* and 3*F* 1.2 *ec*.

Both cytological and genetic evidence indicates that the inserted segment carries the locus *fa*, since the 3*C*7 band has been located in the insertion and since hyperploid flies show mottling in the *fa* locus. Cytological observations indicate that the *fa* locus is not involved in the deficiency. Genetic evidence in support of this assumption is impossible to obtain since a change in the *fa* locus has occurred which behaves genetically as a deficiency. Such changes are not rare in the neighborhood of a breakage point. However, in this case the *fa* locus is not the only one which has been changed, but also the *rst* locus which is represented by the fourth band from the break. In this instance a short region involving not less than four and not more than six bands is affected. We have studied 27 breaks in the same region and this is the only case where reattachment to euchromatin produced a block effect. That suggests that such occurrences are not frequent.

Cytological evidence shows that the inserted piece is one band longer than the deficiency. If it were the reverse the interpretation would have been simple, because the total number of all bands in the rearrangement would have been one less than the total number of bands in the original chromosome, and the loss of one band could easily have been accounted for. However, the total number of bands in the rearrangement is one in excess of the total number in the treated chromosome. This increase in the number of bands could occur if the chromosome were split at the time of the break and if two chromatids were broken at different places. In subsequent rearrangements the longer of the two segments and the chromatid with the shorter deficiency were recovered.

A picture of this situation can be obtained from the diagram in figure 1. On the right side the limits of both the deficiency and the inserted segment

coincide. At the time of breakage the chromosome might not have been split at this point, or if it were split the breaks in both chromonemata must have coincided. However, on the left side the inserted segment is longer than the deficiency and the chromosome must have been split in this region at the time of the break and the breakage points in the two chromonemata did not coincide but were one band apart.

Now, what is the probable mechanism which produced these changes? It is known that the rearrangement involves chromosomes which are brought into the egg by the x-rayed sperm. Therefore the breaks were undoubtedly caused by x-ray treatment. The right break, which occurred

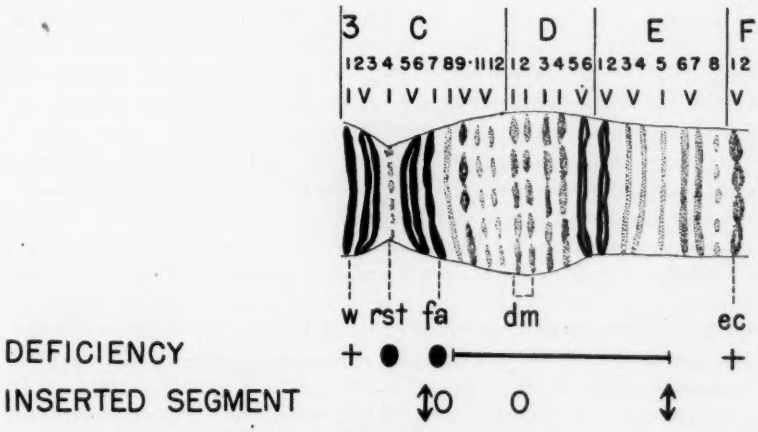


FIGURE 1

Above, section of normal salivary chromosome. Below, changes in the two affected strands: straight line shows extent of deficiency, + = unaffected gene, full 0 = gene showing mutation; in the other strand, arrows show position of breaks, 0 = gene producing mottled effect.

at the same point in both of the recovered strands, might have been caused (*a*) by a single electron hit causing a break in the chromonema before it was split, (*b*) by a single electron hit causing breaks at the same point in two strands, or (*c*) by two independent hits each causing a break in one of the two strands at approximately the same point. The left breaks, which are at different points in the two recovered chromatids, might have been caused by (*a*) two independent hits, each producing a break in one of the two chromatids of the chromonema, or (*b*) one hit producing two breaks, one in each chromatid, at two different points of the chromonema.

Genetic analysis of the region adjacent to the left break gives the clue for deciding between two possibilities. It is evident from figure 1 that in

the strand involving a deficiency the loci *fa* and *rst* are affected, namely, that a section of the chromosome adjacent to the break and extending through at least four bands has been changed. It is known that a break in the chromosome sometimes induces a change in the locus adjacent to that break. We have a few cases where a locus a short distance from the break has been affected, presumably through the position effect. However, such a condition is very rare and seems to be specific to the locus. Except for the present case it has never been observed for the *rst* locus. The incidence of breaks induced in the two strands at different places but close together is not known, but since this is the first case of this type on record it is justifiable to assume that it must be low. Since the frequency of each of these two events is low it seems very probable that their occurrence together indicates that they were induced by the same cause, which in the present case means by a single electron hit. This could be brought about through the action of a single excitation shock given by an electron to the surrounding matter and diffused over a relatively large area covering two strands in width and four bands or about 1.5 microns in length. However, this effect took place in the sperm where the chromosomes are very likely coiled and thus the length of the affected region may be considerably smaller than in the salivary gland chromosomes where the chromonema is stretched. It is reasonable to assume that salivary gland chromosomes are about one hundred times as long as chromosomes in the sperm, thus a 1.5 micron section of salivary chromosomes is condensed to about 150 Å in the sperm. The spread of the physical excitation phenomena over such distance is not contrary to physical expectation.

*Summary.*—Cytogenetic analysis of *N*264-86, which was obtained from an x-rayed male, shows that two strands of the *X*-chromosome were affected by the treatment. One of the strands has a deficiency from 3*C*8 to 3*E*5, inclusive, and in addition changes in *fa* and *rst* loci, while a piece from 3*C*7 to 3*E*5, inclusive, has been taken out from the other strand and inserted into chromocenter of 4. Thus the right breaks occurred at the same point, while the left breaks occurred at different points but close to each other. It seems probable that the left breaks and the changes in adjacent loci were induced by the excitation produced by a single electron hit.

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